CD4, CD8 AND BCL2 EXPRESSION IN LICHEN PLANUS AND ITS RELATIONSHIP BETWEEN LYMPHOCYTIC EXOCYTOSIS AND APOPTOSIS

A dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the requirements for the award of the degree of

M.D in PATHOLOGY

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCE & RESEARCH

PEELAMEDU, COIMBATORE- 641 004

TAMILNADU, INDIA

CERTIFICATE

CERTIFICATE

This is to certify that the dissertation work entitled **"CD4, CD8 AND BCL2 EXPRESSION IN LICHEN PLANUS AND ITS RELATIONSHIP BETWEEN LYMPHOCYTIC EXOCYTOSIS AND APOPTOSIS"** submitted by **Dr.S.Sathiya** is a work done by her during the period of study in this department from 2016 to 2018. This work was done under the guidance of **Dr.S.Shanthakumari,** Professor, Department of Pathology, PSGIMS&R.

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CERTIFICATE

This is to certify that the thesis entitled **"CD4, CD8 AND BCL2 EXPRESSION IN LICHEN PLANUS AND ITS RELATIONSHIP BETWEEN LYMPHOCYTIC EXOCYTOSIS AND APOPTOSIS"** submitted by **Dr.S.Sathiya** to The Tamilnadu Dr MGR Medical University, Chennai, for the award of the degree of **Doctor of Medicine in Pathology,** is a bonafide record of research work carried out by her under my guidance. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Dr.S.Shanthakumari.
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DECLARATION

I, **Dr.S.Sathiya** do hereby declare that the thesis entitled **"CD4, CD8 AND BCL2 EXPRESSION IN LICHEN PLANUS AND ITS RELATIONSHIP BETWEEN LYMPHOCYTIC EXOCYTOSIS AND APOPTOSIS"** is a bonafide work done by me under the guidance of **Dr.S.Shanthakumari,** Professor, Department of Pathology, PSG Institute of Medical Sciences & Research. This study was performed at the PSG Institute of Medical Sciences & Research, Coimbatore, under the aegis of The Tamilnadu Dr MGR Medical University, Chennai, as part of the requirement for the award of the MD degree in Pathology.

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To
Dr S Sathiya
Postgraduate
Department of Pathology
Guide: Dr S Shanthakumari
PSG IMS & R
Coimbatore 641 004

Ref: Project No.16/310

Date: October 13, 2016

Dear Dr Sathiya,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 28.09.2016 to conduct the research study entitled "*CD4, CD8 and Bcl2 expression in lichen planus to establish the relationship between lymphocytic exocytosis and apoptosis*" during the IHEC meeting held on 30.09.2016.

The following documents were reviewed and approved:

1. Project submission form
2. Study protocol (Version 1.1 dated 12.10.2016)
3. Confidentiality statement
4. Application for waiver of consent
5. Data collection tool
6. Current CVs of Principal investigator, Co-investigator
7. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 30.09.2016 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr Sudha Ramalingam	MD	Epidemiologist, Ethicist Alt. member-Secretary	Female	Yes	Yes
5	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
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Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented
   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

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September 15, 2017

To
Dr S Sathiya
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Guide: Dr S Shanthakumari
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The Institutional Human Ethics Committee PSG IMS & R, Coimbatore - 4, has reviewed your proposal on 15th September, 2017 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your request to renew the approval for the study entitled:

"CD4, CD8 and Bcl2 expression in lichen planus to establish the relationship between lymphocytic exocytosis and apoptosis"

The following documents were received for review:

1. Request for renewal dated 12.09.2017
2. Status report

After due consideration, the Committee has decided to renew the approval for the above study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
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4	Dr Sudha Ramalingam	MD	Epidemiologist, Ethicist Alt. member-Secretary	Female	Yes	Yes
5	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The approval is valid for one year (13.10.2017 to 12.10.2018).

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member – Secretary
Institutional Human Ethics Committee

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https://en.wikipedia.org/wiki/Lichen_planus
http://www.dermnet.com/dermatology-pictures-skin-disease-pictures/
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Instances where selected sources appear:

12

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ACKNOWLEDGEMENT

I would like to render my sincere prayers to the almighty for what I am today.

"***The journey of a thousand miles begins with one step".***

My first step towards my dissertation was induced by my guide and my mentor **Dr.S.Shanthakumari M.D** Professor, Department of Pathology. Without her, my aim of composing and completing my thesis would not have been a reality. I express my sincere gratitude for her expertise, assistance and patience throughout the process of writing my thesis.

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Family is the biggest pillar in anyone's life. I have no words to express for my parents **Mr. T. Shanmugam & Mrs. S.Kousalya Devi** for sacrificing a lot and never letting me to suffer for anything in my life. Dad and Mom you are the best!

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INTRODUCTION

INTRODUCTION

Lichen Planus is an inflammatory dermatosis involving either mucosal surfaces or epithelium of skin. Clinical presentation of lichen planus is characterized by polygonal and violaceous papules. Histologically it is characterized by,

- Hyperkeratosis
- Wedge shaped hypergranulosis
- Irregular acanthosis
- Basal cell vacoular damage
- Dense subepithelial band like inflammatory infiltrate

The subepithelial band like inflammatory infiltrate is composed of variable proportions of CD4 and CD8 T lymphocytes [1]. The band like inflammatory infiltrate of mononuclear cells in the upper dermis is associated with lymphocytic exocytosis into the epidermis and damage to the epidermal basal cell layer [2].

The main histological feature of lichen planus is the formation of colloid bodies also referred as hyaline, cytoid or Civatte bodies. The ultra structural studies have revealed that colloid bodies are apoptotic keratinocytes [3]. The presence of lymphocytes close to apoptotic epidermal cells suggests a role for a cytotoxic T-cell mediated immune reaction in the pathogenesis of lichen planus.

In apoptosis cell death is mediated by cytotoxic T lymphocytes (CTL) or natural killer (NK) cells. The target cells show rapid nuclear damage and characteristic DNA fragmentation. The mucous membranes involved are the oral and genital mucosa. Very rarely the mucosa of the anus, nose, larynx, conjunctiva and urethra are involved. It is estimated that the prevalence Oral lichen planus varies from 0.5-4% of the general population [4].

Cell mediated immune reactions appear to be important in the pathogenesis of lichen planus. The cellular response initially consists of CD4 lymphocytes. It is also increased in the peripheral blood. In lichen planus, CD8 cells appear to recognize an antigen which is associated with MHC class I and they result in their death by apoptosis.

Bcl-2, a proto-oncogene that protects cells from apoptosis is seen to be increased in lichen planus [5]. It allows a few of the cells to escape apoptosis. This process helps in prolonging the inflammatory process. In both oral and cutaneous lichen planus CD8 cells predominate in the epithelial and sub epithelial compartments. CD4 cells play an important helper role by the secretion of Th1 cytokines.

Therefore this study is proposed to identify the type of lymphocytic infiltrate and its correlation with apoptosis and association of BCL2 with the inflammatory reactions and correlating it with its phenotypic expression.

AIMS & OBJECTIVES

AIM AND OBJECTIVES

- To study the type of lymphocytic infiltrate and its correlation with apoptotic keratinocytes in Lichen planus with the help of IHC markers.
- Expression of BCL2 in Lichen Planus
- And correlating the above findings with phenotypic expression.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Skin is the largest organ in the body. It acts as external protective layer against various external threats including mechanical stress , ultraviolet light, water loss and various chemicals. It also provides moisture control and prevents excessive water loss. It resists fungal and bacterial invasion. It's rich in Langerhans cells which are antigen presenting cells and when the skin is breached an immune response against these antigens are initiated [6].

Melanin provides protection against ultraviolet (UV) radiation. Synthesis of vitamin D3 (cholecalciferol) is done by the action of UV light on 7-dehydrocholestrol. Cholecalciferol is further processed to produce the active agent 1, 25-dihydroxycholecalciferol. It's important in calcium and bone formation.

Skin also plays a vital role in thermoregulation. It's the largest sensory organ in the body. It has different receptors for touch, pressure, pain and temperature. Hair and nails are specialised components of skin. The overall appearance of skin plays an important role both clinically as an important indicator of health and human behaviour.

Skin diseases can occur due to various reasons. Added to the diseases, injuries are also common in the form lacerations, cuts and boils.

SKIN STRUCTURE:

Skin has three main layers,

- Epidermis
- Dermis
- Sub cutis/Hypodermis/Panniculus

EPIDERMIS:

It's a highly specialised self-regenerating stratified squamous epithelium which produces a protein, keratin. Keratin is a tough layer and is protective and partially water resistant. Epidermis also contains melanocytes which produces melanin [6]. Melanin protects against UV light. Langerhans cell acts as antigen presenting cells and Merkel cell act as touch receptors. Rete ridges extend downwards into the superficial dermis, and provide resistance to frictional shearing forces. The epidermis comprises the following cell types,

- Keratinocytes
- Melanocytes
- Langerhans cells
- Merkel cells

EMBRYOLOGY OF KERATINOCYTES: [7]

The epidermis develops as a single layer of ectodermal cells and by five weeks of gestation epidermis will differentiate focally into two layers – the basal layer or stratum germinativum and the overlying periderm. By ten weeks an

intervening layer the stratum intermedium develops. At nineteen weeks of gestation there are several layers of intermediate cells. The peridermal layer of cells starts to flatten. By 23 weeks the keratinisation is fully developed within the stratum intermedium, at this juncture, most of the periderm cells have shed, and the keratinizing cells represent the newly formed stratum corneum. [8]

By 6-7 weeks of gestation immature desmosomes, early hemidesmosomes and distinct basement membrane of the epidermis are formed. At the end of first trimester structurally mature basement membrane is formed. Numerous microvilli and cytoplasmic micro vesicles are displayed by the surface cells of the periderm. It increases the area in contact with amniotic fluid and the active interchange between the periderm cells and the amniotic fluid.

Till sixteen weeks the tonofilaments are sparse and then develops as dense accumulations. These are observed in cells of the intermediate layer as evidence of beginning of keratinisation. [8]

Layers of the epidermis includes,

- Stratum basalis (Basal cell layer)
- Stratum spinosum(Squamous cell layer)
- Stratum granulosum(Granular layer)
- Stratum lucidum
- Stratum corneum(Horny layer)

STRATUM BASALIS:

It contains single layer of cells, ovoid in contour and lie with their long axes perpendicular to the underlying basement membrane. The cells have more basophilic cytoplasm often contain melanin pigment and contain dark-staining round to oval nuclei. The cells are connected with the overlying squamous cells by intercellular bridges united by desmosomes. The base is attached to the subepidermal basement membrane by modified desmosomes, termed hemidesmosomes. The mitotic activity is noted in the basal cell layer. [9]

STRATUM SPINOSUM:

The cells are polyhedral in shape and overlie the stratum basalis and form a mosaic with 5 to 10 layers thick. Towards the skin surface they became flattened. The intercellular bridges separate the cells and are united by desmosomes. Hyalouranic acid an important component of glycosaminoglycans is quite abundant in the matrix between keratinocytes and occurs predominantly in the spinous layer.

Glycocalyx is a gel like substance which provides cohesion between the epidermal cells and on other hand allows the rapid passage of water soluble substances through the intercellular spaces and helps in individual cell movements.

Tonofilaments are seen within the cytoplasm of the keratinocytes of the stratum spinosum. These are loose bundles of electron dense filaments and each measures 7 to 8 nm in diameter. The electron microscopy of desmosomes

consists of two dense plaques of 10-15nm in thickness. Cadherins are derived from multiple genes and represents calcium dependant cell adhesion molecules. Desmosomal cadherins are desmogleins and desmocollins. These molecules are attached to intracytoplasmic intermediate filaments by plakoglobin and desmoplakin.

Auto antibodies to desmogleins 1 and 3 results in clinical blisters due to loss of cell-cell adherence [10-11] .A noted correlation has been established between desmoglein expression in normal skin and pathologic lesions in which specific desmogliens are disrupted with regard to the plane of blister formation due to loss of cell-cell adhesion.

STRATUM GRANULOSUM:

The granular layer consists of flattened cells. The cytoplasm of the cell is filled with keratohyaline granules that are deeply basophilic and irregular in size and shape. The thickness of the granular layer is proportional to the thickness of horny layer. The granular layer is three to four layers thick when the horny layer is thin. In palms and soles the horny layer is very thick and hence the granular layer has ten layers in thickness.

The mature keratin forming transitional zone of the epidermis is represented by the granular cell layer. In contrast to the stratum basalis and stratum spinosum, lysosomal enzymes are present in abundance in granular layer. The lysosomal enzymes play important role in the autolytic changes occurring in the granular layer. [12]

STRATUM LUCIDUM:

It's an additional layer seen predominantly in the palms and soles.

STRATUM CORNEUM:

Stratum corneum is composed of anucleate cell layer. The horny layer stains eosinophilic. On formalin fixation the horny layer becomes shrunken and results in characteristic basket weave architecture in routine sections. Glutraldehyde fixation causes precipitation of the formalin –soluble substances within the horny cells and allows the staining of the contents of the horny layer. Fluorescent staining shows the cells of the horny layer are arranged in orderly vertical stacks. [13]

IMMUNE FUNCTIONS OF KERATINOCYTES:

Keratinocytes produces interleukins, colony stimulating factors (CSFs), Interferons, Tumour necrosis factor, Transforming growth factors (TGFS) and Platelet derived growth factor. Accordingly these keratinocytes may play an active role in elaboration of molecular signals that facilitate lymphocyte homing and activation. [7]

IMMUNOHISTOCHEMISTRY:

Keratin consists of two families of intermediate filament proteins, type 1 and type 2 members. The type1family of intermediate filament proteins are located on chromosome 17q21.2 and type2 family of intermediate proteins are located on chromosome 12q13.13. Different keratins are expressed by

different layers of the human epidermis. Keratin5 and keratin 14 are predominantly expressed by the basal cells.

Supra basal keratinocytes express keratin 1 and keratin 10. Keratin 2 is expressed by terminally differentiated keratinocytes. Keratin 9 is expressed by the supra basal keratinocytes in palmar and plantar epidermis.

One should be familiar with expression of keratin by normal structures because specific mutations are associated with different keratins. p63 a member of p53 gene is implicated in both the development and maintenance of stratified epithelial tissues, including the epidermis.

p63 expression is seen in basal/supra basal cells of the epidermis, outer root sheath and hair matrix cells of the hair follicle, basal cells situated in the outermost layer of sebaceous glands. p63 is over expressed in squamous cell carcinomas and basal cell carcinomas and it's a marker for poorly differentiated squamous cell carcinoma. [14]

MELANOCYTES [7]

Melanin-synthesizing dendritic cells are melanocytes which are located within the basal layer of the epidermis, hair bulb and outer root sheath of hair follicles. The cell contains round to oval, dark stained nuclei and are smaller than the basal keratinocytes and has a clear halo of surrounding cytoplasm which is a result of shrinkage of the cells during processing.

Melanin is transferred by means of dendritic processes from the melanocytes to the basal keratinocytes. Greater amount of melanin is noted in the basal keratinocytes than in the melanocytes.

SPECIAL STAINS:

In Haematoxylin-Eosin stained sections melanocytes may be difficult to appreciate and there are special stains that can facilitate their detection by light microscopy. Silver stains indicate the presence of melanin. It contains both argyrophilic and argentaffin stains.

Argyrophilia is defined as the ability of melanin to be impregnated with silver nitrate solutions and on reduction with hydroquinone to silver, it stains black. Melanin may be bleached by a strong oxidizing agent, such as hydrogen peroxide or potassium permanganate and is used in heavily melanized tumours where the pigments may obscure the nuclear details. [14]

IMMUNOHISTOCHEMISTRY:

Immunohistochemical detection of melanocytes has been classically and most commonly accomplished by polyclonal antibodies to S100 proteins. These are family of calcium binding proteins [15]. In skin addition to melanocytes S100 labels Langerhans cells, specialised macrophages, Schwann cells, sweat glands and adipocytes. HMB-45 and additional reagents such as monoclonal antibodies to Melan-A/MART-1, tyrosinase and PNL2 are newly established.

MERKEL CELLS:

These cells are seen within the basal cell layer of the epidermis, oral mucosa, and the bulge region of the hair follicles [16]. Merkel cells may represent the rudimentary touch receptors.

LANGERHANS CELLS:

Langerhans cell appear in the epidermis by 7 weeks of gestation These are bone marrow derived, dendritic, antigen presenting cells. Langerhan cells express CD1a reactivity. Electron microscopy of the Langerhan cells exhibits a markedly folded nucleus with the absence of Tonofilaments. Birbeck or Langerhans granules are seen in the cytoplasm of the Langerhan cells. These granules have the highly characteristic appearance of a tennis racquet.

DERMIS:

The dermis adjacent to the epidermis is called the papillary dermis. Papillary dermis contains relatively more collagen fibres and contains numerous blood vessels, sensory nerve endings and sensory structures. The reticular dermis is the deeper tough layer of horizontally arranged collagen and elastic fibres with fibroblasts. The cellular components of dermis consist of – fibroblasts, dermal dendritic cells, macrophages, mast cells [7]. The basement membrane at the junction of epidermis and dermis is known as the dermo-epidermal junction.

SUBCUTIS:

The deepest layer is the subcutis also called the panniculus or hypodermis. This layer is composed of adipose tissue often compartmentalised by fibrous septa, extending downwards from dermis to the underlying structural connective tissue fascia. The dermis and subcutis contain an assortment of skin adenexa (appendages) such as hair follicles, sebaceous glands, eccrine (sweat) glands and ducts.

LICHENOID INTERFACE DERMATITIS: [17]

Two types of inflammatory infiltrate are noted,

- Lichenoid interface dermatitis
- Vacoular interface dermatitis

Lichenoid interface dermatitis is defined by the following two patterns at the basement membrane zone. The destruction of the basal keratinocytes and a superficial, band like lymphocytic inflammatory infiltrate of varying density closely approximating the epidermis. The cytoplasm of the altered basal keratinocytes becomes brightly eosinophilic .The nucleus which is at first pyknotic is later extruded so that round to oval eosinophilic bodies are found in the lower epidermis and upper papillary dermis. These structures are known as Colloid or cytoid bodies. With the regeneration of the keratinocytes the basement membrane zone takes on a disorderly irregular appearance.

A lymphocytic infiltrate is present in the superficial dermis adjacent to the altered keratinocytes. The lymphocytic infiltrate is moderate to dense and melanophages may be present in the underlying papillary dermis (pigment incontinence).

Lichenoid interface alteration overlaps with the vacoular epidermal interface alteration. Dyskeratosis predominates in the lichenoid pattern whereas vacuolization of the basal keratinocytes is the hall mark of the vacoular pattern.

LICHEN PLANUS:

Lichen planus (LP) is an immune mediated and chronic inflammatory disease that affects the skin, hair, nails and mucous membranes. Most common presentation is the involvement of cutaneous flexor surfaces of the extremities and they present with small itchy violaceous papules in the middle-aged adults. The 6 “P’s” of lichen planus are “Pruritic, Purple, Polygonal, Planar, Plaques and Papules”. [18]

EPIDEMIOLOGY:

The incidence of lichen planus varies between 0.22% and 5% of the adult population worldwide [19-20]. Lichen planus in India the incidence is about 0.38%. Oral lichen planus seems to be more frequent with a reported incidence of 1 to 4%. Lichen planus is rare in children and commonly affects adults during their fourth to sixth decade.

There is no obvious racial predisposition for lichen planus but a study from UK found that children originating from the Indian subcontinent represented 80.8% with lichen planus.

Case reports in both adults and children have shown association between lichen planus and the following, [21]

- Chronic liver diseases such as chronic active hepatitis
- Primary biliary cirrhosis
- Complication of hepatitis B vaccination
- Viral and bacterial antigens
- Trauma
- Metal ions and medications.

CLINICAL PRESENTATION:

The lesions are characterized by small, flat-topped, shiny, polygonal, violaceous papules that may coalesce into papules. Networks of white lines are often seen in the papules known as Wickham striae. Pruritus is usually seen. The disease has a predilection for the flexor surfaces of the fore arms, legs and glans penis. Mucosal involvement of the lesion is noted in the oesophagus and oral cavity. Lichen planus can rarely manifest in the larynx and conjunctiva.

Lichen planus can involve various sites and they are named as,

- Cutaneous lichen planus
- Nail lichen planus

- Oral lichen planus
- Vulvovaginal lichen planus
- Esophageal lichen planus
- Ocular lichen planus
- Laryngeal lichen planus

CUTANEOUS LICHEN PLANUS: [22]

It has different clinical subtypes based on the morphology of the lesions and site of involvement.

Sub types based on morphology of the lesions:

1. Classic (papular) lichen planus
2. Hypertrophic lichen planus
3. Vesiculobullous lichen planus
4. Actinic lichen planus
5. Annular lichen planus
6. Atrophic lichen planus
7. Linear lichen planus
8. Follicular lichen planus
9. Lichen planus pigmentosus and
10. Lichen planus pigmentosus –inversus

CLASSIC LICHEN PLANUS:

It present as a shiny, red/purple-colored, flat–topped papule. Adherent transparent and thin scaly lesions are noted. The surface of well-developed papules shows a fine whitish points or lacy lines known as Wickham's striae [23]

HYPERTROPHIC LICHEN PLANUS:

Hypertrophic lesions are usually confined to the shins, and sometimes they are more generalized. It is characterized by hyperkeratotic thick pruritic red brown- to purple –gray plaques that involves the anterior region of the legs and interphalangeal joints. Cutaneous horns, keratoacanthoma and verrucous carcinoma may also develop in hypertrophic lichen planus. The main lesion may be surrounded by polygonal papules. [24]

VESICULOBULLOUS LICHEN PLANUS:

Bullous form of lichen planus is a rare variant of lichen planus and is characterized by development of vesico-bullous lesions. Fewer cases have been reported in the literature. Extensive vacoular change of the basal cell layer is the common etiology of bullae formation in Bullous lichen planus. [25]

A familial form of Bullous lichen planus has been reported and is associated with younger age of onset, increased severity, lengthened duration of the disease. Familial forms may inherit an autosomal dominant pattern and display variable penetrance.BLP typically presents as tense bullae on top of typical violaceous polygonal LP lesions. Dorsal aspects of the hands and feet as

well as the trunk are involved. The most common site is the legs. Treatment of BLP is similar to that of other variants.

ACTINIC LICHEN PLANUS:

It's a rare type of lichen planus. It presents as nummular patches or plaques with a hypo pigmented halo around a hyperpigmented center. It's more prevalent among the African Americans, Indians and Middle Eastern peoples. It predominantly affects the sun exposed areas. [26]

ANNULAR LICHEN PLANUS:

Annular lichen planus is one of the uncommon forms of lichen planus. It classically involves the male genitalia (glans penis and penile shaft), axilla, groin and extremities. Oral and genital lesions have been reported in annular lichen planus.The majority of patients' show central clearing with a purple to white annular edge. The lesions range in size from 0.5 to 2.5 cm in diameter. Annular forms are usually asymptomatic especially when arising in the genital area.

An increased reporting of lesions that are both atrophic and annular has occurred in the literature and new variant atrophic annular lichen planus has been coined.

ATROPHIC LICHEN PLANUS:

Atrophic lichen planus occurs in areas that are previously affected by other LP variants. The anatomical distribution of the lesions may helpful in diagnosis of atrophic lichen planus because the anatomical distribution of the

lesions may resemble that of hypertrophic or chronic annular lichen planus. The long term use of super potent topical corticosteroids may predispose the patient to develop atrophic lichen planus.

Histopathological examination reveals the epidermis appears thin and there is loss of normal rete ridges and the inflammatory infiltrate is usually less than typical lichen planus.

LINEAR LICHEN PLANUS:

Linear form of lichen planus occurs at the site of healed herpes zoster lesions. It is also known as zosteriform lichen planus if it presents in a dermatomal pattern. The lesions follow the lines of Blaschko. Linearly oriented lichen planus can also occur in Koebner phenomenon, but this pattern is not considered as true linear form. Linear lichen planus lesions are unilateral, pruritic and may involve any area of the body. [27]

It also been reported in association with hepatitis C infection, HIV infection and metastatic carcinoma. Differential diagnosis include,

- Inflammatory linear verrucous epidermal nevus
- Lichen striatus
- Linear psoriasis
- Linear Darier-White disease

Biopsy from the involved lesion shows histopathological features identical to classic lichen planus.

FOLLICULAR LICHEN PLANUS:

Follicular lichen planus (Lichen planopilaris) is a variant of lichen planus with keratotic follicular lesions associated with common manifestations of lichen planus. Middle aged women and men are more commonly affected. Rare cases have been reported in the children .Pin point hyperkeratotic papules are often found on the scalp and it frequently affects the vertex but can also involve other parts of the scalp [27]. The keratotic follicular lesions and associated erythema are best seen at the margins of the scarring alopecia

The Graham Little-Piccardi-Lassueur syndrome is a closely related rare entity in which there is cicatricial alopecia of the scalp, follicular keratotic lesions of glabrous skin and variable alopecia of the axillae and groins. It s most commonly seen with androgen insensitivity syndrome (testicular feminization)

"Fibrosing alopecia in a pattern distribution" is a suggested subtype that has a centroparietal pattern Fibrosing frontal alopecia is more commonly seen in the postmenopausal women. The Graham Little-Piccardi-Lassueur is characterized by the triad of follicular-based spinous papules on the body, scalp or both. It is preferentially more common in females

The treatment of choice is topical corticosteroid therapy and intralesional steroids. Oral hydroxychloroquinine and tetracycline's appear to be more effective. Hair transplants and scalp reductions can be used in end-stage diseases.

LICHENPLANUS PIGMENTOSIS:

Erythema dyschromicum perstans (lichen planus pigmentosus) is a slow growing, asymptomatic, ash colored or brown macular hyperpigmented lesion. The lesion is more commonly seen in the trunk and upper limbs. Periorbital hyperpigmentation is a rare presentation of the disease.

The term Erythema dyschromicum perstans should be used when the lesions have or had previously an erythematous border, whereas 'ashy dermatosis' should be used for other cases without this feature. Erythema dyschromicum perstans is also regarded as macular variant of lichen planus. This condition is also seen associated with HIV and HCV infection. It is most commonly seen in Indians and dark skinned individuals [5].

NAIL LICHEN PLANUS:

Nail involvement is the common manifestation of disseminated lichen planus and it involves up to 10% of patients with lichen planus lesions involving other sites [28]**.** Nail lichen planus usually appears during the fifth or sixth decades of life and affects both the genders equally. [29]

Finger nails are more commonly involved than the toe nails. Affected nails presents with,

- Longitudinal ridges
- Pitting
- Onychorrhexis

- Distal splitting and
- Brown discoloration

Biopsy of the involved tissue shows classic histopathological features of lichen planus. Dermoscopy helps in the evaluation of disease progress and prognosis as changes in the nail matrix, nail bed and perionychium can be observed using this technique. [28]

The early manifestations include pitting of the nail matrix and trachyonychia, while advanced diseases show chromonychia, lamina fragmentation, onycholysis and splinter haemorrhage. The differential diagnosis includes psoriasis, onychomycosis and nail manifested alopecia areata. Untreated nail lichen planus progress to anonychia.

Treatment includes topical steroid and systemic steroid therapy with oral prednisolone or intramuscular injection of triamcinolone acetonide has provided better outcomes. Inspite of the treatment recurrence of the lesions are more common. [30]

ORAL LICHEN PLANUS:

Oral lichen planus can be seen involving the buccal and glossal mucosa and most of them often consist of a lacy reticular network of white coalescent papules. The common aggravating factors of the lesion involves,

- Stress
- Contact allergy to metals

- Food flavourings
- Spicy foods
- Poor oral hygiene

Oral lichen planus is a relatively common mucosal disease that can present isolated or associated with cutaneous lichen planus. It is estimated that prevalence of oral lichen planus varies from 0.5 to 4% of the general population. It is more commonly prevalent in females.

Oral lichen planus has several clinical subtypes,

1. Reticular
2. Erosive
3. Papular
4. Plaque –like
5. Bullous subtypes

The most common site involved in the oral lichen planus is the buccal mucosa and it comprises of 80-90% of oral lichen planus cases. The Koebner phenomenon is not only present in cutaneous lichen planus but also occur in the setting of oral lichen planus.

RETICULAR ORAL LICHEN PLANUS:

It is the most common type of oral lichen planus. It is usually asymptomatic and it is often diagnosed during routine oral examination. Macroscopically they are characterized by white lacy streaks surrounded by

well-defined erythematous borders. This type of pattern is less evident on the dorsum of the tongue [31].

PAPULAR ORAL LICHEN PLANUS:

Papular type of oral lichen planus is characterized by small white pinpoint papules that can be easily missed as they are small and asymptomatic. These types of lesion are noted in the initial and transient phases of the oral lichen planus [32].

PLAQUE –LIKE ORAL LICHEN PLANUS:

Large homogenous white patches are characteristic. Leukoplakia and plaque like lesions have similar clinical presentation. Hence leukoplakia should be ruled out before making a diagnosis of plaque like oral lichen planus. Plaques like lesions are more commonly noted in tobacco smokers and they are always associated with poor prognosis. Reoccurrences of the lesions are not noted in plaque like lesions.

EROSIVE ORAL LICHEN PLANUS:

Clinically erosive oral lichen planus can present as atrophic or erythematous ulcerations and mucosal ulcerations. Faint radiating striae are noted in these types of lesions. The ulcers are seen to be covered by a pseudo membrane. Erosive oral lesions have a multi focal pattern of distribution.

Multi focal pattern of distribution is important because the lesions can be quite painful. It has a negative impact over the patient's quality of life. The

symptoms may range from discomfort to severe painful episodes. Dorsum of the tongue is most commonly involved and it causes dysgeusia.

ATROPHIC ORAL LICHEN PLANUS:

Atrophic oral lichen planus has similar presentations of erosive oral lichen planus. Atrophic lesions on a background of erythema and radiating white striae are noted. Gingiva is primarily affected in atrophic OLP [33]. Buccal mucosa particularly in the Posteroinferior areas adjacent to the second and third molar teeth is also involved by these lesions. Some experts combine the two entities and name it atrophic and erosive lichen planus.

BULLOUS ORAL LICHEN PLANUS:

Bullous lesions are not common in oral cavity. In rare instances Bullous lesions can also be noted in oral cavity.

VULVOVAGINAL LICHEN PLANUS:

Most commonly seen in peri menopausal or post menopausal women. Children are rarely affected [34]. Vulvovaginal lichen planus has a similar pattern to oral lichen planus.

It has three main sub types,

- Erosive vulvovaginal lichen planus
- Papulosquamous vulvovaginal lichen planus
- Hypertrophic vulvovaginal lichen planus

EROSIVE VULVOVAGINAL LICHEN PLANUS:

It is the most type of lichen planus that affects the mucosal membranes of the vagina [35] .The most significant sequelae of chronic erosive lichen planus is the formation of scar tissue. It causes,

- Resorption of the labia minora and clitoral hood
- Clitoral burying (68%)
- Stenosis of the introitus (59%)
- Total obliteration of the vagina

Scarring and adhesions of the vagina may interfere in the sexual intercourse.

In a study done by A.L.O Helgesen et al stated that 46 out of 58 patients with erosive lichen planus has complain of sexual abstinence and dyspareunia. [36]. This study was done in a specialised centre in Norway over a period of 7 years. In addition to dyspareunia vulvar lichen planus also causes intense pruritus with chronic vaginal discharge, burning, and postcoital bleeding.

PAPULOSQUAMOUS AND HYPERTROPHIC LICHEN PLANUS:

Both Papulosquamous and hypertrophic lichen planus has a similar presentation of oral lichen planus.43-100% of vulvar cases may have concomitant oral involvement, whereas about 25% of oral lichen planus patients may have vulvar involvement. The coexistence of oral and genital lesions is

known as vulvovaginal-gingival syndrome. Skin lesions are as frequent as 17%-22% in this syndrome.

OESOPHAGEAL LICHEN PLANUS:

Oesophageal lichen planus may be accompanied by other mucosal lesions or less commonly concomitant with cutaneous lesions. Lichen planus of oesophagus is a rare presentation. Extra oesophageal lesions can be found in almost all patients with oesophageal lichen planus. 90% of cases show proximal oesophageal involvement with or without distal involvement. Thyroid dysfunction is the most common associated disorder [37]. Superficial gastritis is also noted with oesophageal lichen planus. Upper GI endoscopy should be considered in patients with complaints of dysphagia, odynophagia, weight loss or other oesophageal symptoms.

OCCULAR LICHEN PLANUS:

Lichen planus has a significant involvement of the ocular region. The lesions that are caused by ocular lichen planus are,

- Blepharitis
- Mild to moderate xerophthalmia
- Cicatricial conjunctivitis
- Keratouveitis
- Keratoconjunctivitis sicca
- Punctuate epithelial erosions

- Corneal ulceration/ scarring
- Ocular surface squamous neoplasia

Conjunctival involvement starts as a streak involving the palpebral conjunctiva. In a case series of 9 lichen planus patients with ophthalmological signs, 7 cases had vulvovaginal-gingival syndrome. All patients developed subepithelial fibrosis and lacrimal duct stenosis.

Biopsy for histopathology and immunofluroscence studies is the only way to differentiate ocular lichen planus from other causes of irreversible scarring Keratoconjunctivitis. Fragmented and shaggy sub epithelial fibrinogen layer in the conjunctiva is indicative of lichen planus. It is essential to diagnose and treat such disease quickly and efficiently to avoid the dire consequences of blindness.

LARYNGEAL LICHEN PLANUS:

Involvement of lichen planus in larynx is rare. Only few cases have been reported with the involvement in larynx. The lesion is seen involving the epiglottis region than other parts of the larynx. There is no involvement of vocal cords are noted.

NORMAL IMMUNE RESPONSE: [38]

Lichen planus is thought to be a T- cell mediated autoimmune disease and it mainly targets the basal keratinocytes, which can be triggered by a variety of situations including viruses, drugs and contact allergens.

Immunity is defined as the protective mechanism which protects the body from infectious pathogens. The classification of immunity falls into two broad categories,

- Innate immunity (also called natural , or native immunity)
- Adaptive immunity (also called acquired, or specific immunity)

INNATE IMMUNITY:

Innate immunity is also called natural or native immunity which is always present ready to provide defense against microbes and to eliminate damaged cells.

COMPONENTS OF INNATE IMMUNITY:

- Epithelial barriers: Epithelia of the skin, GIT and respiratory tracts provide mechanical barriers to the entry of microbes. Antimicrobial molecules such as defensins and lymphocytes located in the epithelia combat microbes at the affected site. If microbes breach epithelial boundaries other defense mechanisms comes into play.
- Phagocytic cells: Monocytes and neutrophils are phagocytes that are present in the blood and that can be rapidly recruited to any site of infection. Monocytes that enter the tissue and mature are called as macrophages.
- Dendritic cells: These are specialized cell population present in epithelia, lymphoid organs and most tissues. These capture the protein antigens and present it to the T lymphocytes. Dendritic cells are endowed with a rich

collection of receptors that sense the microbes and play a role in inflammation and anti-viral defense.

- Natural killer cells: Provides early protection against many viruses and intra cellular bacteria.
- Other cell types such as mast cells, several plasma proteins and proteins of the complement system are involved in the innate immunity.

The cells present in innate immunity are capable of recognizing certain microbial components that are shared among the related microbes. These microbes are often essential for infectivity. Two types of molecular patterns are noted which include pathogen-associated molecular patterns and damage associated molecular patterns.

The cellular compartments have the pattern recognition receptors. Plasma membrane receptor detects extra cellular microbes, Endosomal receptors detect ingested microbes and cytosolic receptor detects microbes in the cytoplasm. The receptors involved are,

- Toll –like receptors
- NOD- like receptors
- C-type Lectin receptors
- RIG-like receptors
- G protein – coupled receptors
- Mannose receptors

Reactions of innate immunity are done by two mechanisms,

1. Inflammation: Cytokines and other complement activated proteins trigger the vascular and cellular components of inflammation. The recruited leucocytes destroy the microbes and ingest and eliminate the damaged cell.
2. Antiviral defense: Type 1 interferon's produced in response to viruses act upon the infected cell and degrades viral nucleic acids and inhibits viral replication. This state is called antiviral state.

ADAPTIVE IMMUNITY:

The adaptive immune system consists of lymphocytes and their products including antibodies. There are two types of adaptive immunity:

- Humoral immunity
- Cell mediated or cellular immunity

Humoral immunity is mediated by B-lymphocytes and their secreted products, known as antibodies (also called Immunoglobulins). Cell mediated immunity is mediated by T-lymphocytes. Both classes of lymphocytes express highly specific receptors for a wide variety of substances, which are called antigens.

In lymphoid organs different classes of lymphocytes are noted. Mature lymphocytes that have not encountered with antigens are called as naive lymphocytes. After encounting an antigen they became activated and are known

as effector cells. Effector cells eliminates the microbes, whereas memory cells live in a state of heightened awareness and are able to react rapidly and strongly in case of reinfection.

T-LYMPHOCYTES:

There are three major populations of T cells are noted. They serve distinct functions, they are,

- Helper T-lymphocytes
- Cytotoxic T-lymphocytes
- Regulatory T- lymphocytes

T-lymphocytes develop in the thymus from precursors that arise from hematopoietic stem cells. 60-70% mature T-cells are found in the blood. Through T cell receptor, specific cell bound antigens are identified. T-cell receptors consist of a disulfide –linked heterodimer made up of α and β polypeptide chain. The αβ TCR recognizes peptide antigens that are presented by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells. Limitations of specificity of T cells for peptides by MHC molecules are called as MHC restriction.

Each TCR molecules are non- covalently linked to six polypeptide chains. They form the CD3 complex and the zeta proteins are invariant in all T cells. They are involved in the transduction of signals into the T cell that are triggered

by binding of antigen to the TCR. Together TCR and the proteins form a TCR complex.

Small population of T cells expresses another type of TCR composed of gamma and delta polypeptide chains. The gamma delta TCR recognises the peptides, lipids and small molecules without requirement of the MHC proteins. The epithelia of skin mucosa of the gastrointestinal and urogenital tracts are protected against the microbes by the gamma delta complex.

NK-T cells express a very limited diversity of TCRs and they recognize the glycolipids that are displayed by the MHC like molecule CD1. CD4 and CD8 are expressed on two mutually exclusive subsets of αβ T cells. Approximately 60% of mature T cells are CD4 and 30% are CD8. CD4 T lymphocytes functions as cytokine secreting helper cells that assist macrophages and B lymphocytes to combat infections.

Most CD8 T lymphocytes functions as cytotoxic T lymphocytes also known as killer cells. CD4 and CD8 T lymphocytes serve as coreceptors in T-cell activation. During antigen recognition CD4 molecules bind to class II MHC molecules that are displaying the antigen and CD8 molecules bind to class I MHC molecules. CD4 and CD8 coreceptor initiates the signals and activates the T cells.

CD4 helper T cells can recognize and respond to antigen displayed only by class II MHC molecules, whereas CD8 cytotoxic T cells recognize cell-bound antigens only in association with class I MHC molecules. Integrins are adhesion

molecules that promote the attachment of the T cells to antigen presenting cells. Additional signals provided by antigen presenting cells are needed, in which CD 28 plays an important role.

B- LYMPHOCYTES:

These are the only cells in the body capable of producing antibody molecules. The antibody molecules are the mediators of humoral immunity. The B lymphocytes develop from the precursors in the bone marrow. The mature B cell constitutes about 10% to 20% of the circulating peripheral lymphocyte population. The cells are also present in peripheral lymphoid tissues such as lymph nodes, spleen and mucosa associated lymphoid tissue.

Membrane bound antibodies of the IgM and IgD are present on the surface of all mature, naive B cells. After stimulation by antigen and other signals B cells develop into plasma cells. A single plasma cell can secrete hundreds to thousands of antibody molecules per second. Antibody secreting cells are also detected in the peripheral blood and these are called as plasmablasts.

Igα and Igβ are essential for signal transduction through the antigen receptors. Other molecules include the type 2 complement receptors (CR2 or CD21) which recognize complement products generated during innate immune responses to microbes and CD 40, which receives signals from the helper T cells. CR2 is also used by the Epstein - Barr virus as a receptor to enter and infect B cells.

Functions of each lymphocytes:

B lymphocyte → Neutralization of microbe, Phagocytosis and Complement activation

Helper T lymphocyte → Activation of macrophages Inflammation and activation of T and B lymphocytes

Cytotoxic T lymphocyte → killing of infected cell

Regulatory T lymphocyte → suppression of immune response

Natural killer cell → killing of infected cell

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) MOLECULES:

MHC molecules display the peptide fragments of protein antigens for recognition by antigen specific T cells. MHC molecules are fundamental to the recognition of antigens by T cells and are linked to many auto immune disorders. In humans MHC molecules are called human leukocyte antigens (HLA). Initially these molecules were noted on the leukocytes by the binding of antibodies.

The genes encoding the HLA molecules are clustered on a small segment of chromosome 6. On the basis of their structure and cellular distribution and function, MHC gene products are classified into two major classes,

- Class I MHC molecules
- Class II MHC molecules

CLASS I MHC MOLECULES:

These molecules are expressed on all nucleated cells and platelets. They are heterodimers consisting of a polymorphic α or heavy chain linked non-covalently to a smaller non polymorphic protein called β2 microglobulin. The α chains are encoded by three genes and are designated as,

- HLA-A
- HLA-B and
- HLA-C

These chains are lie close to one another in the MHC locus. The extra cellular region of the α chain is divided into three domains α1, α2 and α3. α1 and α2 forms a groove or cleft where the peptides bind.

Class I MHC molecules display peptides that are derived from proteins, such as viral and tumour antigens. These antigens are located in the cytoplasm and are usually produced in the cell and class I peptides are identified by the CD8 T lymphocytes.

- The cytoplasmic proteins are degraded in proteasomes and the peptides are transported into the endoplasmic reticulum. There these molecules are recognized by class I molecules and the peptides bind to it.
- Peptide loaded MHC molecules along with β2 – microglobulin form a stable trimer and it is transported to the cell surface. CD8 molecules

bind to the α3 domain and therefore the peptide –class I complexes are identified by CD8 T cells and these exhibit cytotoxic actions.

- CD8 T cells are called as CLASS I MHC-RESTRICTED cells. Because CD8 cells recognize the peptides only if presented as a complex with class I MHC molecules.
- One of the important functions of CD8 CTLs is to eliminate the viruses which may infect any nucleated cell, and tumour cells which may arise from the nucleated cells.
- All nucleated cells express class I HLA molecules and can be surveyed by CD8 T cells.

CLASS II MHC MOLECULES:

Class II MHC molecules are encoded in a region called HLA-D. HLA-D has three sub regions,

- HLA-DP
- HLA-DQ
- HLA-DR

Each molecule has a α and β chains. The extra cellular regions of both α and β chains have two domains and are designated as α1 ,α2 and β1 and β2. Like class I molecules, class II molecules also have peptide binding clefts facing outwards. This cleft is formed by interaction of the α1 and β1 domains.

- Class II molecules present antigens that are derived from extracellular microbes and soluble proteins. The proteins are proteolytically digested and the peptides form a complex with the MHC molecules. The class II β2 domain has a binding site for the CD4 cells
- CD4 T cells can recognize the antigens only in the context of self class II molecules; they are referred to as class II MHC restricted.
- Class II molecules are mainly expressed on cells that present ingested antigens and respond to T-cell help. The cells mainly involved are the macrophages, B lymphocytes and dendritic cells.
- Cytokines, tumour necrosis factor and lymphotoxin also encode in the region of MHC locus.

The combination of HLA alleles in each individual is called the HLA haplotype. Each individual inherits one set of HLA genes from each parent and thus typically express two different molecules for every locus. No two individuals (other than identical twins) are likely to express the same MHC molecules. Therefore grafts exchanged between these individuals are recognized as foreign bodies and are attacked by the immune system.

A number of auto immune and other diseases are associated with the inheritance of particular HLA alleles.

ACTIVATION OF T LYMPHOCYTES:

The naive T lymphocytes are activated by antigen and costimulators in peripheral lymphoid organs and proliferate and differentiate into effector cells that migrate to any site where the antigen is present. Secretion of the cytokine IL-2 is the earliest response of the CD4 helper T cells. High affinity receptors of IL-2 are also expressed.

The functions of helper T cells are mediated by the combined actions of CD40-ligand and cytokines. When CD4 helper T cells recognize antigens, the T cells express CD40 ligand, which engages on the macrophages or B cells and activates these cells. Activated CD4 T cells differentiate into effector cells that secrete different sets of cytokines and perform different functions.

- Cells of T_H1 subset secrete the cytokine IFN-gamma, and it is a potent activator of macrophage. The combination of CD40 and IFN-gamma results in classical macrophage activation which leads to microbial destruction.
- T_H2 cells produce IL-4 and this stimulates B cells to differentiate into IgE – s secreting plasma cells and IL5 activates eosinophils. Eosinophils and mast cells bind to IgE coated microbes such as helminthic parasites and function to eliminate helminths.
- T_H2 cells also induce the alternative pathway of macrophage activation and this is associated with tissue repair and fibrosis.

- T_H17 cells secrete IL-17 which helps in the recruitment of neutrophils and monocytes which destroy some extra cellular bacteria and fungi.
- Activated CD8 T lymphocytes differentiate into CTLs that kills the microbes and eliminate the microbial reservoirs.

ACTIVATION OF B LYMPHOCYTES:

On activation the B lymphocytes proliferate and then differentiate into plasma cells that secrete different classes of antibodies with distinct functions. Antibody responses depend upon the T cell help and are said to be T-dependent. The B cells ingest protein antigens into vesicles degrade them and they are bound to Class II MHC molecules for recognition of the T-helper cells.

The activated T cells express CD40 ligand and secrete cytokines and they stimulate the B cells. T-independent responses are relatively simple. Each plasma cell is derived from an antigen stimulated B cell and secretes antibodies. Polysaccharides and lipids stimulate secretion mainly of IgM antibody. Isotype switching is induced by cytokines including IFN-gamma and IL-4.

Activated B lymphocytes migrate into the follicles and form germinal centres, which are the major site of isotype switching and affinity maturation. The T cells which help in the process are called as follicular helper T-Cells.

- Secreted antibodies bind to microbes and prevent them from infecting the cells and neutralize the effect of microbes.
- IgG antibodies coat microbes and target them for Phagocytosis.

- IgG and IgM activate the complement system by classical pathway and promotes Phagocytosis.
- IgA is secreted from the mucosal epithelia and neutralises microbes in the lumens of the respiratory and gastrointestinal tracts.
- IgG is actively transported across the placenta and provides immunity to the new born until the immune system becomes mature.
- IgE and eosinophils kills parasites mainly by release of eosinophilic granule which provides toxic effect to the microbes.

IgG antibodies have half-life of about three weeks. The plasma cells generated in germinal centres, migrate to the bone marrow and live for months or even years and produces antibodies.

Decline of immune responses: The effector lymphocyte induced by an infectious pathogen die by apoptosis and returns to the immune system in a resting phase. The memory cells are expanded pool of antigen-specific lymphocytes and they respond faster and more effectively when reexposed to the antigen. The generation of memory cells is an important goal of vaccination.

APOPTOSIS:

Apoptosis is a pathway of cell death that is induced by a tightly regulated program in which the cells destined to die which activate intrinsic enzymes that degrade the cells own nuclear DNA and nuclear and cytoplasmic proteins. The

cells are broken into fragments and become tasty targets for phagocytes. Apoptosis is referred to as programmed cell death.

CAUSES OF APOPTOSIS:

- Apoptosis in physiologic situations
- Apoptosis in pathologic conditions

APOPTOSIS IN PHYSIOLOGIC SITUATIONS:

Death by apoptosis is a normal phenomenon and it serves to eliminate the cells that are no longer needed. Apoptosis can be seen in physiologic conditions such as,

- During embryogenesis the destruction of cells take place including implantation, organogenesis, developmental involution and metamorphosis.
- Involution of hormone- dependent tissues upon hormone withdrawal such as endometrial tissue, ovarian follicular atresia in menopause and regression of the lactating breast after weaning.
- Cell loss in proliferating cell populations and death of host cells such as neutrophils after acute inflammatory response and lymphocytes at the end of an immature response.

APOPTOSIS IN PATHOLOGIC CONDITIONS:

Apoptosis takes place in pathologic conditions such as,

- DNA damage: Radiation, cytotoxic anticancer drugs and hypoxia can damage DNA, either directly or via production of free radicals. Intrinsic mechanisms are triggered that induce the apoptosis.
- Accumulation of misfolded proteins: Improperly folded proteins may lead to stress of endoplasmic reticulum, and it culminates in apoptotic cell death.
- Cell death in infections: This type of cell death is noted in the viral infections, T-cell mediated immunity which leads to cell death in tumours and cellular rejections of transplants.
- Pathologic atrophy in organs after duct obstruction such as occurs in the pancreas, parotid gland and kidney.

MORPHOLOGIC AND BIOCHEMICAL CHANGES IN APOPTOSIS:

Morphologic features seen with the electron microscope includes,

- Cell shrinkage
- Chromatin condensation
- Formation of cytoplasmic blebs and apoptotic bodies
- Phagocytosis of apoptotic cells or cell bodies, usually by macrophages

MECHANISM OF APOPTOSIS:

Caspases are the enzymes on activation leads to apoptosis. Caspases are cysteine proteases that cleave proteins after aspartic residues. The process of apoptosis may be divided into

- Initiation phase – Caspase become catalytically active
- Execution phase – Caspase trigger the degradation of critical cellular components.

Two pathways converge on Caspase activation,

- Intrinsic (mitochondrial) pathway of apoptosis
- Extrinsic (Death receptor – initiated) pathway of apoptosis

INTRINSIC (MITOCHONDRIAL) PATHWAY OF APOPTOSIS:

Mitochondrial pathway is the major mechanism of apoptosis in all mammalian cells. The release of mitochondrial pro-apoptotic proteins is tightly controlled by the BCL2 family of proteins. The BCL family are divided into three groups based on their pro-apoptotic or anti-apoptotic function, they are

- Anti-apoptotic
- Pro-apoptotic
- Sensors

ANTI-APOPTOTIC:

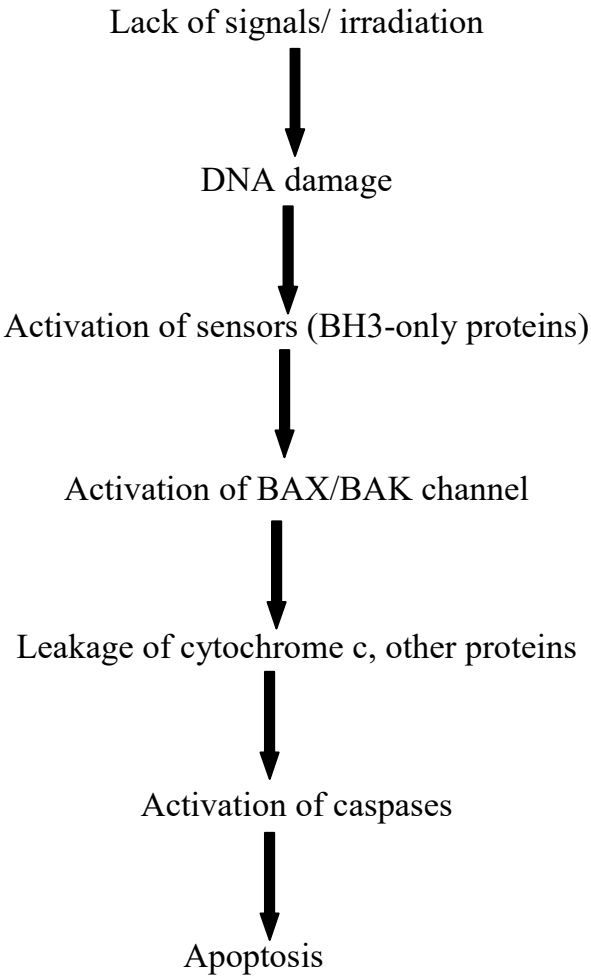
BCL2, BCL-XL and MCL1 are the principal members of this group, they possess four BH domains. These proteins reside in the outer mitochondrial membranes as well as cytosol and ER membranes. The outer membrane is impermeable and they prevent leakage of cytochrome c and other death-inducing proteins into the cytosol.

PRO-APOPTOTIC:

BAX and BAK are the two pro-apoptotic members of the group. These also have four BH domains. BAX and BAK oligomerize within the outer mitochondrial protein and promote mitochondrial outer membrane permeability. This forms a channel and leads to outer mitochondrial membrane, allowing leakage of cytochrome c from the intermembranous space.

SENSORS:

Members of this group include BAD, BIM, BID, Puma and Noxa. This group of members has only one BH domain. The domain are present on BH3, hence they are called BH3-only proteins. BH3-only proteins act as sensors of cellular stress and damage and regulate the balance between the other two groups.



BCL2 prevents the leakage of death inducing proteins from the outer mitochondrial membrane. The damaged or misfolded cells are sensitized by the sensor BH3 cells. These sensors in turn activate two critical effectors, BAX and BAK. This causes isomerisation and forms oligomers that insert into the mitochondrial membrane and causes leakage of the proteins from inner mitochondrial membrane to leak out into the cytoplasm.

On releasing into the cytosol, cytochrome c binds to a protein called APAF-1 (apoptosis-activating factor-1), which forms apoptosome. This complex binds to caspase-9, activates the pro-caspases and the active enzymes which mediates the execution phase of apoptosis.

EXTRINSIC (DEATH RECEPTOR- INITIATED) PATHWAY:

Engagement of plasma membrane death receptors on a variety of cells activates this pathway. Death domains are cytoplasmic domain that is involved in protein-protein interactions and they are essential for delivering apoptotic signals. The death receptors belong to the TNF receptor family. The best known death receptors are the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95).

The ligand for Fas is called Fas ligand (FasL). It is expressed on T cells that recognize self antigens and on some cytotoxic T lymphocytes. When FasL binds to Fas, three or more molecules of Fas are brought together and their cytoplasmic death domain and is called FADD (Fas-associated death domain). FADD again binds to an inactive form of caspase-8, and caspase activation leads to active caspase-8.

This pathway of apoptosis can be inhibited by the FLIP protein which binds to pro-caspase-8. The extrinsic and intrinsic pathways of apoptosis involve fundamentally different molecules for their initiation but there can be interconnections. Two initiating pathways converge to a cascade of caspase activation, which mediates the final phase of apoptosis.

The mitochondrial pathway leads to the activation of initiator caspase -9and the death receptor pathway initiates the initiator caspase 8 and 10. The formation of apoptotic bodies break cell into bite-sized fragments that are edible for phagocytes. In healthy cells, phosphatidylserine is present on the inner leaflet

of the plasma membrane, but in apoptotic cells this Phospholipid flips out and expressed on the outer layer of the membrane where it is recognized by several macrophage receptors.

Cells that are dying by apoptosis secrete soluble factors that recruit phagocytes. Few apoptotic bodies are coated by thrombospondin, an adhesive glycoprotein that is recognized by the macrophages and phagocytes target the dead cells for engulfment. Apoptotic bodies also coated with natural antibodies and proteins of the complement system notably C1q and are recognized by the phagocytes.

Hence numerous receptors on phagocytes and ligands induced on apoptotic cells serve as eat me signals and are involved in the binding and engulfment of these cells. This process of apoptotic cells is so efficient that dead cells disappear, often within minutes without leaving a trace and signs of inflammation.

PATHOGENESIS OF LICHEN PLANUS:

The pathogenesis of lichen planus can be triggered by,

- Immunopathology
- Organisms
- Genetics
- Environmental factors

IMMUNOPATHOLOGY:

The cells commonly included in Immunopathology of lichen planus are;

- Langerhan cells
- Chemokines
- Keratinocytes

CD8 T cells form the predominant population of the lymphocytic infiltrate with a significant proportion of Delta-gamma T cells [39].PCR studies in Lichen planus potentially suggest the presence of auto antigens. In lichen planus the IHC and electron microscopic examination revealed an increased number of Langerhans cells.Gene expression studies demonstrated an up-regulation of type-1 interferon –inducible genes and the presence of a specific chemokine signature in lichen planus. CXCL9, the ligand of the receptor CXCR3, was significantly increased in the study.

In lichen planus both CD4 and CD8 T cells accumulate in the dermis, whereas CD8 T cells infiltrate the epidermis. The CD8 cytotoxic T cells recognize an antigen associated with major histocompatibility complex (MHC) class 1 on lesional keratinocytes and lyse them. [40].

The number of Langerhans cells in the epidermis is increased in the disease very early. It is predominantly seen near the keratinocytes expressing HLA-DR antigen. Close contact of lymphocytes with Langerhans cell and macrophages are noted by immunoelectron studies. Specific conjugations

between CD4 T lymphocytes and dendritic cells, between CD8 T cells and degenerated basal keratinocytes are noted in the oral epithelium.

ROLE OF CHEMOKINES AND KERATINOCYTES:

The chemokines helps in recruitment and activation of cytotoxic TH1 cells and plasmacytoid dendritic cells. T cells secrete RANTES which trigger mast cell degranulation with simultaneous release of tumour necrosis factor α(TNF-α). This in turn regulates lesional T-cell RANTES secretion. This mechanism contributes to increase T cell infiltration and disease progression. [41]

The keratinocytes are type I interferon producers which leads to skin lesions. Lichen planus shows increased CD1a Langerhan cells and Factor XIIIa+ cells [42].These cells act as antigen presenting cells. Intercellular adhesion molecule 1(ICAM-1) and vascular cell adhesion molecule 1(VCAM-1) are elevated in lichen planus. The basement membrane disruption is contributed by Mast cell degranulation and T cell secretion of metalloproteinase 9(MMP-9). Bone morphogenic protein-4 (BMP-4) over expression contributes to apoptosis of epithelial cells in oral LP. Dysregulation of MYC transcription factor is involved in malignant transformation. [43].

The infiltrating cells are predominantly T lymphocytes with very few B lymphocytes. Identification of various subtypes of T lymphocytes has given contradictory results with regard to predominance of CD4 helper T cells and CD8 suppressor –cytotoxic T lymphocytes. Both subsets participate in the immunologic reaction.

In the epidermis adjacent to the infiltrate, basal keratinocytes express HLA-DR surface antigen and ICAM-1, both of which are implicated in the interaction between the lymphocytes and their epidermal targets. This phenomenon leads to destruction of the keratinocytes. Immunophenotyping studies on T lymphocytes from the specimen of lichen planus have shown that the majority of clones were CD8 T lymphocytes.

Cell –cell interactions suggest that a cell mediated immune mechanism is operated. Recent studies show that lesional type 1 Interferons produced by plasmacytoid dendritic cells play an important role in chronic cytotoxic inflammation of lichen planus by recruiting cytotoxic effector T lymphocytes via IP10/CXCR3 interactions.

Endothelial- leukocyte interaction is mediated by the P-selectin, an adhesion molecule present within the endothelial cells. Serum levels of P-selectin are increased in lichen planus. The serum level of IL-17 is seen to be increased in the atrophic and erosive type of lichen planus.

Inspite of severely damaged basal cells there is continuous cell proliferation are noted. The Eosinophilia of the keratinocytes and increase in thickness of the granular and cornified layers suggest a decreased epidermal turn over.

CAUSATIVE ORGANISM:

Several studies have suggested, Japanese and Mediterranean population presented with lichen planus are associated with Hepatitis C virus (HCV) [44].

Hepatitis B virus (HBV), Human herpes virus-6 (HHV-6) and HHV-7 and Varicella zoster are the viruses which have been involved in the pathogenesis of lichen planus. Various vaccines induces LP, typically hepatitis B virus vaccines have been shown in some patients to trigger LP. It occurs after second injection of the vaccine.

GENETICS:

Idiopathic LP is more susceptible for genetic causes. Familial cases have been reported and a familial incidence of 10.7% was quoted in one series [45]. LP has also been reported in monozygotic twins. Lichen planus associated with HLA-A3 and HLA-5 has been documented. HLA-A28 in Jewish people with LP and carbohydrate intolerance is also noted in many cases.

Idiopathic LP and mucosal LP may have a different pathogenesis due to genetic heterogeneity.

ENVIRONMENTAL FACTORS:

Most common environmental factors involved in the pathogenesis of lichen planus includes,

- Drugs
- Dental amalgam
- Betel nut
- Chemical exposure
- Miscellaneous

DRUGS:

Variety of drugs causes LP. The terms LP-like eruptions or lichenoid eruption are used in the context of an adverse drug reaction with features of LP. The list of drugs involved in causing lichen planus and LP-like eruptions includes,

- Antimicrobials
- Antihypertensives
- Antimalarials
- Anti depressants
- Diuretics
- Metals
- NSAIDs (non steroidal anti inflammatory drugs) and
- Intravenous immunoglobulin's

DENTAL AMALGUM:

Causative antigen in oral LP is mercury in dental amalgam [46]. Oral lichenoid lesions are often associated with the use of dental amalgam, more studies shows association of mercury in dental amalgam causes oral lichen planus.

BETEL NUT:

Social use of the betel nut is relatively common in India and South East Asia. Betel leaf with mixture of areca nut is associated with oral lichen planus. [47].

CHEMICAL EXPOSURE:

Lichen planus like eruptions were reported in 25% of persons. This population of people has been exposed to chemicals found in colour developer. Two types of reactions were observed – acute (eczematous) and sub acute (lichenoid). LP like lesions have developed on sites exposed to Methacrylic acid esters used in the car industry. Dimethylfumarate which can be found in the sofas can also causes LP like lesions.

MISCELLANEOUS:

Lichen planus has also been associated with radiotherapy and the lesion is confined to the radiation field. Anxiety, depression and stress are common in patients with lichen planus. These may acts as a risk factor for the development of the disease.

PATHOGENESIS OF ORAL LICHEN PLANUS:

Antigen specificity in OLP:

The lymphocytic infiltrate in oral lichen planus is composed of T-cells and the majority of the T cells within the epithelium and adjacent to damaged basal keratinocytes are activated CD8 lymphocytes. In recent studies the majority of sub epithelial and intra epithelial lymphocytes in OLP were CD8 cells [49]. The CD8 T cells are accompanied with apoptotic keratinocytes in OLP lesions.

The majority of cytotoxic clones in lichen planus lesions were CD8 and the majority of nontoxic clones were CD4 T cells. The cytotoxic activity of CD8 lesional T cell clones was partially blocked with anti-MHC class 1 monoclonal antibody. Following antigen recognition and activation CD8 T cells may trigger the keratinocyte apoptosis.

The cytotoxic T lymphocytes may release chemokines that attract additional lymphocytes and other immune cells into the developing OLP. In slightly more advanced lesions, lymphocytes were seen within the lower epidermis and at this stage there was evidence of epithelial damage including vacoular alteration of basal keratinocytes and slight Spongiosis in the spinous zone. These findings are consistent with the current hypothesis that intra epithelial CD8 T cells trigger keratinocyte apoptosis in OLP.

IDENTITY AND LOCATION OF THE LICHEN PLANUS ANTIGEN:

The lichen planus antigen is not known. The role of autoimmunity in disease pathogenesis is supported by many autoimmune features of OLP including disease chronicity, adult onset, female predilection, association with other auto immune diseases, occasional tissue-type association, depressed immune suppressor activity in oral lichen planus patients and the presence of auto-cytotoxic T-cell clones in lichen planus lesions. An early event in lichen planus lesions formation may be keratinocyte antigen expression or unmasking at the future lesion site induced by systemic drugs, contact allergens in dental materials or toothpastes. Subsequently intra epithelial CD8 cytotoxic T-cells

recognize the lichen planus antigen associated with MHC class 1 on lesional keratinocytes and trigger keratinocyte apoptosis.

While the majority of intra-epithelial lymphocytes in OLP are CD8 cytotoxic T cells most lymphocytes in the lamina propria are CD4 helper T cells. An early event in OLP lesion formation may be MHC class II antigen presentation to CD4 helper T cells, followed by keratinocyte apoptosis triggered by CD8 cytotoxic T-cells.MHC class II antigen presentation in OLP may be mediated by Langerhans cells (LC) or keratinocytes.

Keratinocytes in OLP also express MHC class II antigens. CD8 cytotoxic T cells may be activated by the combination of (i) antigen associated with MHC class 1 on basal keratinocytes, (ii) Th1 CD4 T-cell – derived IL-2 and IFN-gamma. Activated CD8 cytotoxic T cells may then trigger basal keratinocyte apoptosis in OLP. Local production of IFN-gamma may maintain keratinocyte MHC class II expression,

On analysis data suggest that many antigen-specific mechanisms may be involved in the pathogenesis of OLP, including

- MHC class-I and MHC class- II restricted antigen presentation by lesional keratinocytes
- Activation of antigen-specific CD4 helper T cells and CD8 helper T cells
- Clonal expansion of antigen-specific T cells and
- Keratinocyte apoptosis triggered by antigen-specific CD8 cytotoxic T cells

Many studies shows that intra epithelial T cells in OLP expressed the naive T-cell marker CD45RA

NON-SPECIFIC MECHANISMS IN OLP:

Many non-specific mechanisms may be involved in the pathogenesis of OLP, including,

1) Mast cell chemotaxis and degranulation stimulated by T-cell RANTES
2) Endothelial cell adhesion molecule expression stimulated by mast cell TNF-α
3) T-cell MMP-9 activation by mast cell chymase
4) Epithelial basement membrane disruption by mast cell proteases or T-cell MMP-9
5) Keratinocyte apoptosis triggered by epithelial basement membrane disruption
6) Intra –epithelial CD8 T cell migration through basement membrane breaks
7) Inflammatory cell survival prolonged by T-cell RANTES and
8) Non-specific T-cell recruitment by keratinocyte – derived chemokines.

Non specific T-cells in OLP may contribute to disease pathogenesis by secreting RANTES and MMP-9 although this remains to be determined.

KERATINOCYTE APOPTOSIS AND LC MATURATION:

The self antigen presentation depends on the activation state of antigen presenting cells (APC). To stimulate T-cell response dendritic cells (DC) and presumably LCs must undergo a process of terminal determination called "maturation". Dendritic cells and Langerhans cell maturation include inflammatory cytokines (IL-1β and TNFα), expressed by activated T-cells, necrotic cells , nucleotides , reactive oxygen intermediates, neurotransmitters, MMP-9 and bacterial lipopolysaccharide[49].

APC endocytosis of apoptotic cells followed by APC maturation may activate self-reactive CD4 T cells that differentiate into Th1 or Th2 phenotypes and promote cell or antibody – mediated stimulus may determine the outcome of CD4 T cell activation.

HISTOPATHOLOGY:

The microscopic feature of lichen planus exhibits,

- Compact orthokeratosis
- Wedge shaped hypergranulosis
- Irregular acanthosis
- Vacuolar alteration of the basal layer
- Band like dermal lymphocytic infiltrate in close approximation to the epidermis

Acanthosis : An increase in the thickness of the stratum spinosum

Hyperkeratosis : An increase in the thickness of the stratum corneum. Hyperkeratosis may be either orthokeratotic or parakeratotic. Orthokeratotic hyperkeratosis is an exaggeration of the normal pattern of keratinisation (no nuclei are seen in the stratum corneum). In parakeratotic hyperkeratosis nuclei are retained in the stratum corneum.

Exocytosis : Presence of inflammatory cells within the epidermis in association with Spongiosis

Spongiosis : epidermal intracellular edema

Vacoular epidermal interface alteration: destruction of the basal keratinocytes characterized by the presence of intracytoplasmic vacuoles and dyskeratotic keratinocytes. A sparse to mild lymphocytic inflammatory infiltrate is usually present.

Lichen planus, in addition to lichenoid interface change several other features are noted within it. Acanthosis is characteristically present and in some cases it may mimic squamous cell carcinoma (hypertrophic lichen planus). Alternatively there is a variant of atrophic lichen planus where the epidermis appears atrophic. Epidermal hyperkeratosis is a regular feature of lichen planus.

The hyperkeratosis is typically orthokeratotic and the presence of parakeratosis can suggest another lichenoid disease. The epidermal granular layer often shows focal “wedge shaped” hypergranulosis. The irregularity of the

lichenoid interface change results in saw tooth appearance of the basement membrane zone. Artifactual cleft formation (Max-Joseph space) between the epidermis and papillary dermis is common and frank haemorrhagic subepidermal bullae may be seen occasionally.

The inflammatory infiltrate in lichen planus is superficial typically moderate to dense predominantly lymphocytic and displays a band like distribution. Melanophages may be abundant especially in lesions involving darkly pigmented skin.

In lichen planopilaris, the changes occur at the level of follicular infundibulum. Lichen planopilaris frequently leads to follicular destruction that is characterized by perifollicular lymphocytic infiltrates , remnants of the follicular epithelium and naked hair fibres partly engulfed by mononucleated or multinucleated phagocytes in late stage lesions.

ELECTRON MICROSCOPY OF LICHEN PLANUS:

In Lichen planus the basal keratinocytes, desmosomes and hemidesmosomes exhibit degenerative changes. The Tonofilaments in the basal cells are decreased in the early stage and are increased in the later stages. The dermal infiltrate extending to the epidermis, causes damage to the lamina densa such a fragmentation. Duplication and irregular folding of the lamina densa are noted.

Some of the lymphocytes have hyper convoluted nuclei and are indistinguishable from Sezary cells. Necrotic keratinocytes or colloid bodies are

located largely in the papillary dermis. It can also be noted in the lower dermis to a lesser extent. The necrotic keratinocytes consists of aggregates of filament bundles, with each filament measuring approximately 10nm in diameter.

Use of Antikeratin immune sera has resulted in intense staining of the necrotic keratinocytes. Fibrin deposits in the upper dermis are a common finding. The vesicular lesions in lichen planus exhibits cytolysis of basal keratinocytes and therefore the blister cavity is situated below the spinous layer.

DIRECT IMMUNOFLUORESCENCE STUDIES IN LICHEN PLANUS:

The dermal epidermal junction exhibits fibrinogen deposition and appears shaggy. Occasional granular deposits of IgM and linear deposits of C3 are noted. 87% of lichen planus cases show staining of necrotic keratinocytes by the use of immunofluroscence. IgM are usually stained, but often seen with IgA, IgG, C3 and fibrin.

The necrotic keratinocytes present in large numbers and in clusters are suggestive of lichen planus. In lichen planopilaris, direct immunofluroscence shows deposition of IgM or IgA, IgG and rarely C3 at the level of infundibulum and isthmus. The necrotic keratinocytes reacts with anti-IgM antibody. The dermal-epidermal junction is virtually always negative for deposition of immunoreactants.

In lichen planus pemphigoides, direct immunofluroscence of perilesional skin shows the presence of IgG and C3 in a linear arrangement along the basement membrane zone. Circulating IgG auto antibodies are directed against

the Bullous pemphigoid antigen 180 (BP180). It is a transmembrane hemidesmosomal glycoprotein of the basal keratinocytes that spans the lamina lucida.

One study shows that in addition to BP180 antigen, the auto antibodies were also directed against a 200-kD antigen. In overlap syndrome LP/LE, the immunofluroscence method differentiates both by the deposition of Immunoglobulins. Deposition of C3 at the dermal-epidermal junction in a linear granular pattern suggests of cutaneous lichen erythematosus.

INVESTIGATIONS:

Histology is the most useful investigation to confirm the diagnosis of lichen planus. The use of dermoscopy has been found useful in some cases. Wickham's striae can be visualized better with the device. Histology will be routinely obtained to confirm the diagnosis of Lichen planus. The earliest findings involve, increase in epidermal Langerhans cells associated with a superficial perivascular infiltrate of lymphocytes and histiocytes, impinging on the dermal- epidermal junction.

Direct immunofluroscence is also an important tool in the diagnosis of lichen planus.

DISEASE COURSE AND PROGNOSIS:

Few cases evolve rapidly and clear within few weeks. In most cases, the papules eventually flatten after a few months and often replaced by an area of

pigmentation that retains the shape of the papule and persists for months or years. The colour change may be gradual from pink to blue and black.

The residual pigmentation may be intense, especially in dark- skinned races. New papules may form while others are clearing .Some papules persists much longer, enlarge and thicken and develop a roughened surface with a prominent violaceous hue – so called hypertrophic LP. Warty lesions and areas of pigment loss can also be seen.

MANAGEMENT AND TREATMENT:

Treatment of lichen planus depends on the localization, clinical form and severity. The aim of treatment in cutaneous lichen planus is to reduce pruritus and time to resolution. Cutaneous lichen planus can clear spontaneously within 1-2 years. Painful erosive LP may need aggressive and long-term treatments.

Lichen planus involving the nail and scalp may induce scars and genital LP, oesophageal and conjunctival involvement of lichen planus induces stricture and fibrosis. Hence rapid treatment should be given to avoid scaring or a fatal course.

CUTANEOUS LICHEN PLANUS: [50]

The goals of therapy are to improve itching and reduce time to resolution of the lesions. The treatment aspects can be graded into.

- First line treatment
- Second line treatment

- Third line treatment

FIRST LINE TREATMENT:

Topical steroids are the first line treatment for limited cutaneous LP. Very potent corticosteroids (clobetasol proportionate ointment 0.05%) once daily until remission. For hypertrophic lichen planus very potent corticosteroids need to be applied under an occlusive bandage. Oral steroids are the wide spread treatment of LP. Prednisolone 0.5-1mg/kg per day for about 4-6 weeks. Oral prednisolone was more effective in patients than the topical application.

SECOND LINE TREATMENT:

Acitretin is used as second line therapy in cutaneous lichen planus. Another second line option is photo chemotherapy or phototherapy. In a small trial, PUVA used three times weekly on one side of the body was compared with no treatment on the other side of the body. The clearance was observed on the treated side only in 50% of the patients. In a retrospective series, clearance within a mean of 3 months was observed in 70% of patients treated with narrow band UVB therapy. Oral corticosteroids with photo therapy are also a possible second line therapy.

THIRD LINE TREATMENT:

Methotrexate can be used as a third line treatment in cutaneous lichen planus. There are four RCTs assessing griseofulvin, hydroxychloroquinine and sulfasalazine. Because of risk of bias in these trials, adverse reactions are noted

with these drugs. Hence these drugs are not recommended in cutaneous lichen planus.

ORAL LICHEN PLANUS:

The aim of treatment in oral lichen planus is to heal erosive lesions in order to reduce pain and permit normal food intake. Education of the patient should emphasize that oral LP frequently has a chronic course marked by treatment induced remission followed by relapse. Alcohol, tobacco, spicy or acidic foods and drinks should be avoided.

FIRST LINE TREATMENT:

- Symptomatic LP: Very potent corticosteroids three times daily until remission, then maintenance therapy
- Severe erosive LP: Prednisolone 0.5-1mg/kg per day until improvement

SECOND LINE TREATMENT:

- Papular plaque like white forms and in absence of erosive lesions: Topical retinoids twice daily
- Resistant to topical corticosteroids: prednisolone 0.5-1mg/kg per day until improvement

THIRD LINE TREATMENT:

- Cortico-dependent or cortico resistant erosive Lp : Systemic immunosuppressive agents (azathioprine, mycophenolate mofetil, Methotrexate, ciclosporin , topical ciclosporin)

Various other forms of lichen planus is treated with potent corticosteroids. Systemic and topical corticosteroids are preferred.

ASSOCIATED CONDITIONS:

Idiopathic LP has been reported in association with diseases of altered or disturbed immunity including ulcerative colitis, alopecia areata, vitiligo, dermato myositis, and morphoea and lichen sclerosus, SLE, pemphigus and paraneoplastic pemphigus. Lichen planus is also seen associated with thymoma, hypothyroidism, myasthenia gravis, hypogammaglobulinaemia and primary Sclerosing cholangitis.

Lichen planus is also seen associated with diabetes. Lichen planus is also seen in certain tattoo reactions, particularly in those areas where coexisting mercury hypersensitivity to the injected dye.

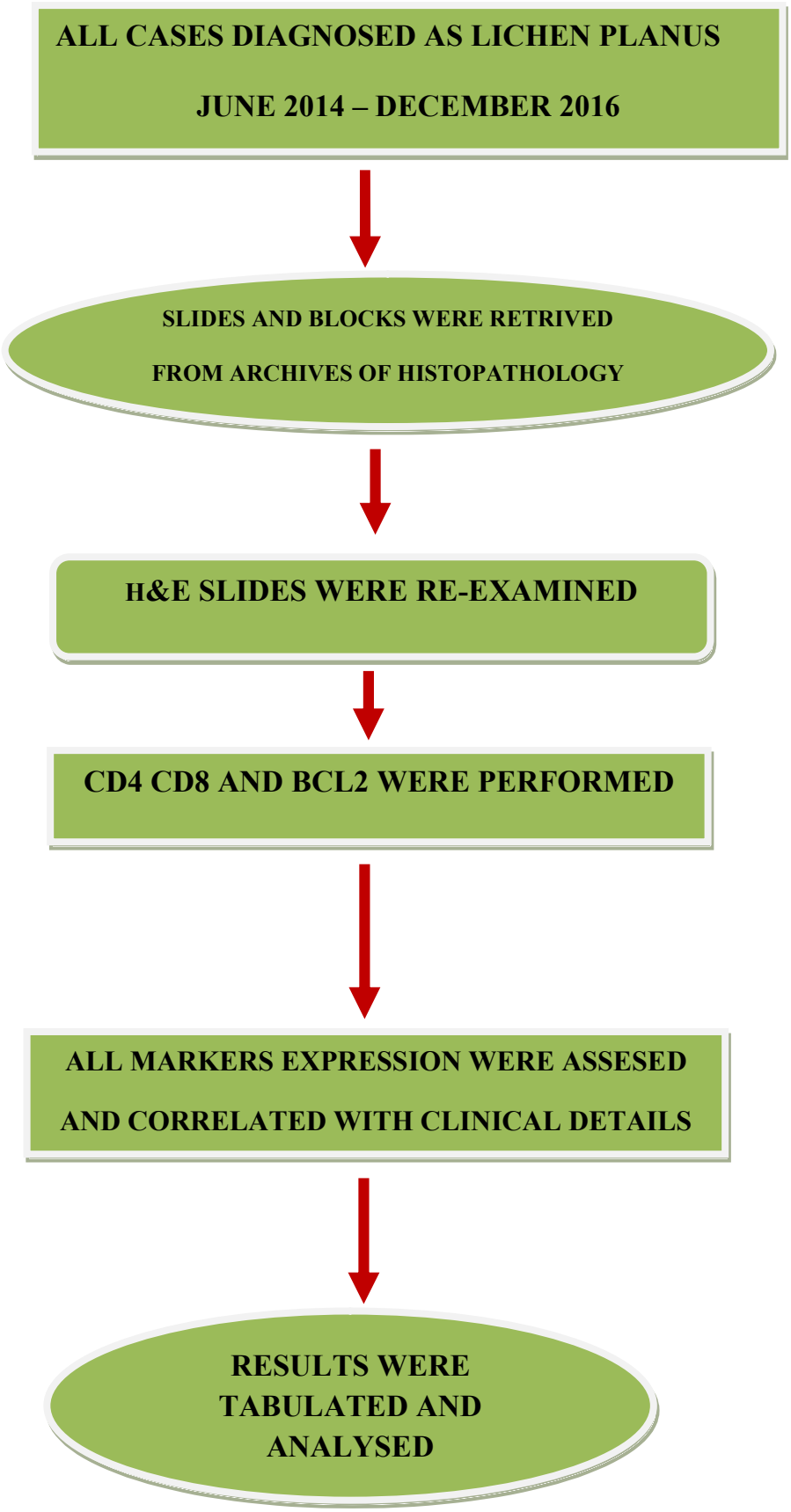
MATERIALS & METHODS

MATERIALS AND METHODS

Cases reported as lichen planus between from June 2014 and December 2016 in the department of pathology of this institute were considered for the study. Samples were included using the following inclusion and exclusion criteria,

- **Inclusion criteria**- Histopathologically diagnosed LP cases (June 2014 – December 2016) were included in the study
- **Exclusion criteria**- The exclusion criteria for the study is inadequate biopsies.
- The clinical details like patient's age, sex, clinical presentation and site of the lesion were taken from the pathology requisition forms and from the medical records department of this institute after obtaining permission from the concerned authorities and institute human ethics committee clearance. Using the above criteria 45 cases were taken for the study. The Hematoxylin and Eosin slides of these cases were analysed for the histologic features.

PLAN OF STUDY:



Immunohistochemistrty was done. The following table shows the clones of various primary antibodies used during this study.

TABLE 1:

THE MARKERS, CLONE OF THE PRIMARY USED:

Antibody	Source	Clonality	Clone	Species	Dilution	Pre-treatment
CD4	Pathinsitu	Monoclonal	EP204	Rabbit	Pre-diluted	None (Normal IHC procedure)
CD8	Pathinsitu	Monoclonal	EP334	Rabbit	Pre-diluted	None (Normal IHC procedure)
BCL2	Pathinsitu	Monoclonal	EP36	Rabbit	Pre-diluted	None (Normal IHC procedure)

The immunohistochemistrty procedure is similar for all markers and is as follows.

PRINCIPLE:

In an Immunohistochemical reaction, the specific antigen present in the tissue is detected in a two stage process

- In the first step, the primary antibody is bound to its targeted epitope in the tissue
- Followed by the detection of bound primary antibody by using secondary antibody by a colorimetric reaction.

The secondary antibody is bound to a dextran polymer with the help of horseradish peroxidase enzyme. The reaction involving binding of secondary antibody with the primary antibody is amplified by the use of a suitable chromogen 3, 3'diaminobenzidine tetra hydrochloride (DAB) and which is responsible for the colour.

STEP 1:

RETIVAL OF THE ANTIGENS:

Antigen site gets obscured by the use of formalin which is used in routine processing of the tissues. The antigen sites are masked by cross linking actions of formalin. The antigen has to be exposed for binding with antibody. So the process by which the antigenic site is exposed is called as antigenic retrieval.

The methods used for this are;

- ❖ Pressure cooker method
- ❖ Microwave method

- Proteolytic digestion method

In our study we used pressure cooker method. In this the antigenic site are unmasked by the action of both pressure and heat. Before this process, the slides were dewaxed and hydrated through graded alcohols. Antigen retrieval by pressure cooker is done for 10 minutes in EDTA buffer at an alkaline PH of 9.

Reagents used are:

- Ethylene Diamine Tetra Acid (EDTA) buffer at an alkaline pH 9.
- 3% Hydrogen peroxide in distilled water.

Hydrogen peroxide blocks the endogenous peroxidase action; therefore it prevents nonspecific background staining.

- A solution of 0.01 M Phosphate Buffered Saline (PBS) is prepared with a PH value of 7.6. The solution is the combination of following substances in a litre of distilled water.
  - Dibasic sodium phosphate, anhydrate 17.5g
  - Monobasic potassium phosphate, anhydrous 2.5g
  - Sodium chloride 17.0g

Blocking agent:

STEP 2:

Primary antibodies against CD4, CD8 and BCL2

STEP 3:

Poly HRP regent – Horse Radish Peroxidase enzyme

STEP 4:

DAB (3, 3'diaminobenzidine tetra hydrochloride) is used as a chromogen and causes permanent coarse brown precipitate

STEP 5:

- Harris Haematoxylin as counter stain
- DPX(Distyrene Phthalate Xylene) – mounting medium

PROCEDURE:

With the help of microtome, 4-5 micron thin sections were cut and fixed on the slides which are coated with Poly L Lysine in a water bath maintained at 37 degree Celsius. The procedure is carried as follows:

- Deparaffinizations of slides by xylene were done.
- Deparaffinised slides were hydrated through graded alcohols
- Washed in running tap water for one minute
- Antigen retrieval is done by using pressure cooker method in EDTA buffer with Ph 9 for 10 minutes
- Fast cooling under tap water
- Washed in PBS buffer at Ph 7.6 for 5 minutes

- The slides are immersed in 0.3% hydrogen peroxide for 20 minutes. It inhibits the background staining due to endogenous peroxidase enzyme.
- The slides are washed with PBS buffer for 5 minutes.
- Slides are incubated in blocking solution for 10 minutes. Blocking solution prevents the non-specific reaction of the reagent antibody with other tissue antigen.
- Slides were washed with PBS buffer three times for 5 minutes
- Slides are incubated with BCL2 primary antibody for one hour
- Wash with PBS buffer three times for 5 minutes
- The sections are covered with super sensitive polymer HRP for 30 minutes
- Wash the slides with PBS three times
- Chromogen DAB is added and incubated for 8 minutes
- Slides are washed with PBS buffer thrice for 5 minutes
- Harris Haematoxylin stain is used as counter stain for one minute
- Washed in running tap water
- Sections are cleared with xylene and mounted with DPX.

PRINCIPLE OF IHC:

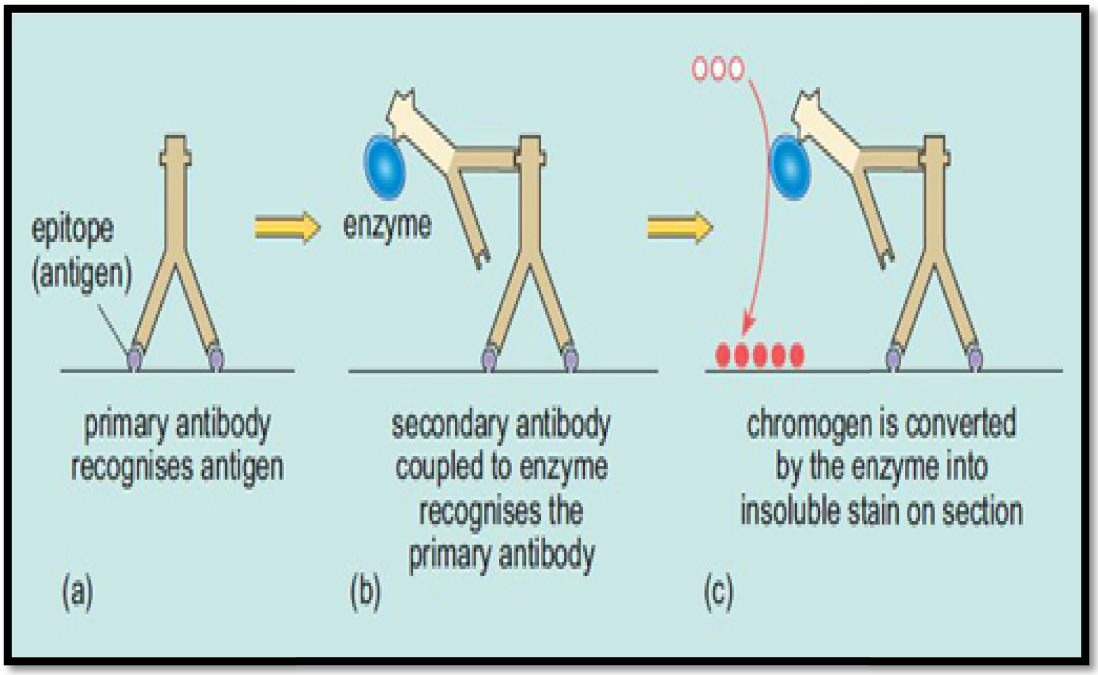
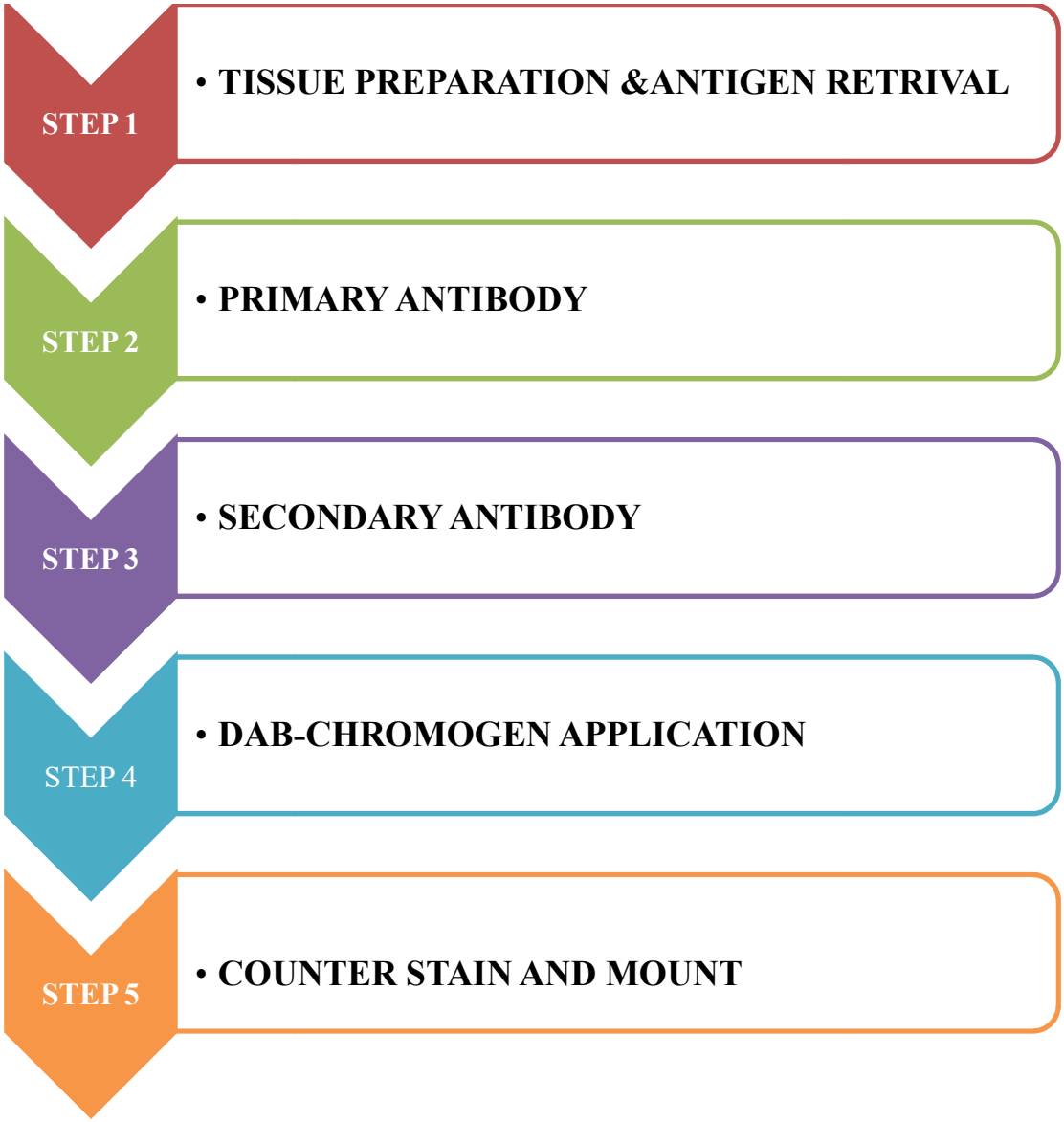


Fig 1: Principles of IHC. Indirect immunolabelling: unlabelled primary antibodies bind to to the antigen on the section. Labelled secondary antibodies bind to the primary antibodies. The secondary antibodies have an enzyme attached which acts on a substrate to deposit a coloured reagent where the antigen is located. (Image adapted from the Open University web)

PROCEDURE:



The stained slides were examined for the expression of CD4, CD8 and BCL2.

RESULTS AND OBSERVATIONS

RESULTS AND OBSERVATION

INCIDENCE:

Department of pathology in our Institute received 15,579 biopsy specimens over a period of two years and six months (June 2014- December 2016) of which 1100 specimens were skin biopsies. 51 cases were lichen planus giving an overall incidence of 4.1%. 45 cases were selected using the inclusion and exclusion criteria. The incidence of lichen planus is as in Chart 1.

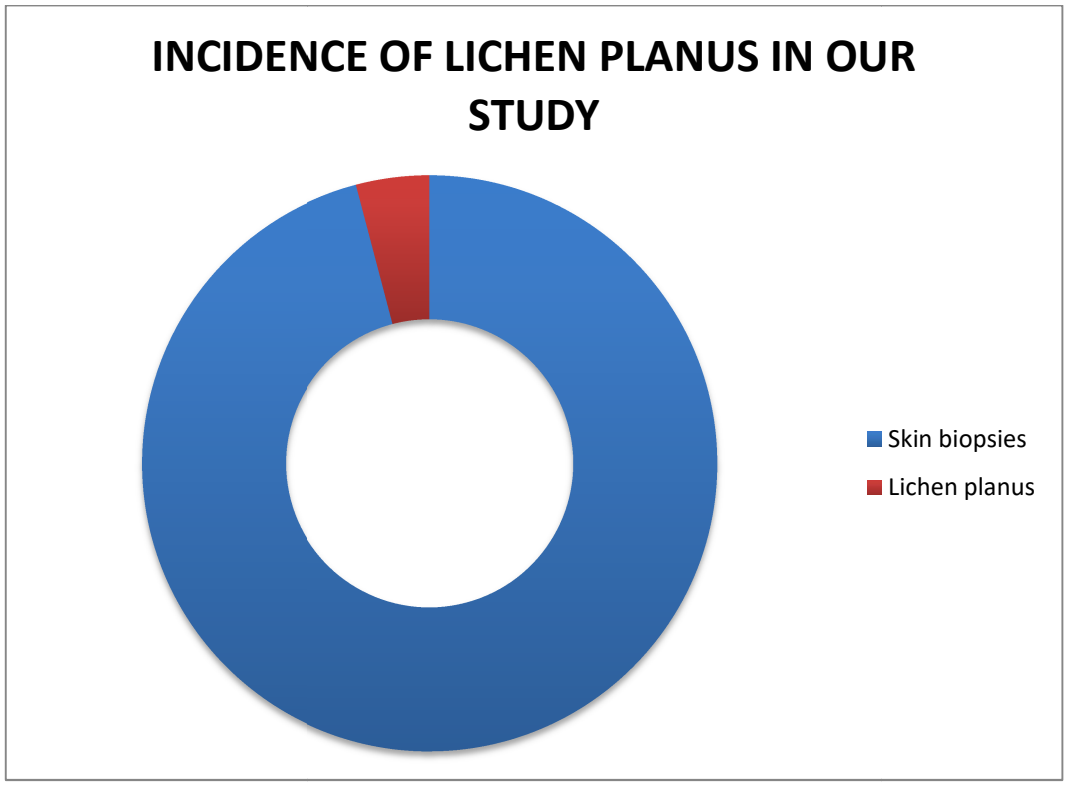


CHART 1: INCIDENCE OF LICHEN PLANUS IN OUR STUDY

AGE DISTRIBUTION :

The most common age of presentation in our study was fourth decade. The age distribution of patients is shown in Chart 2.

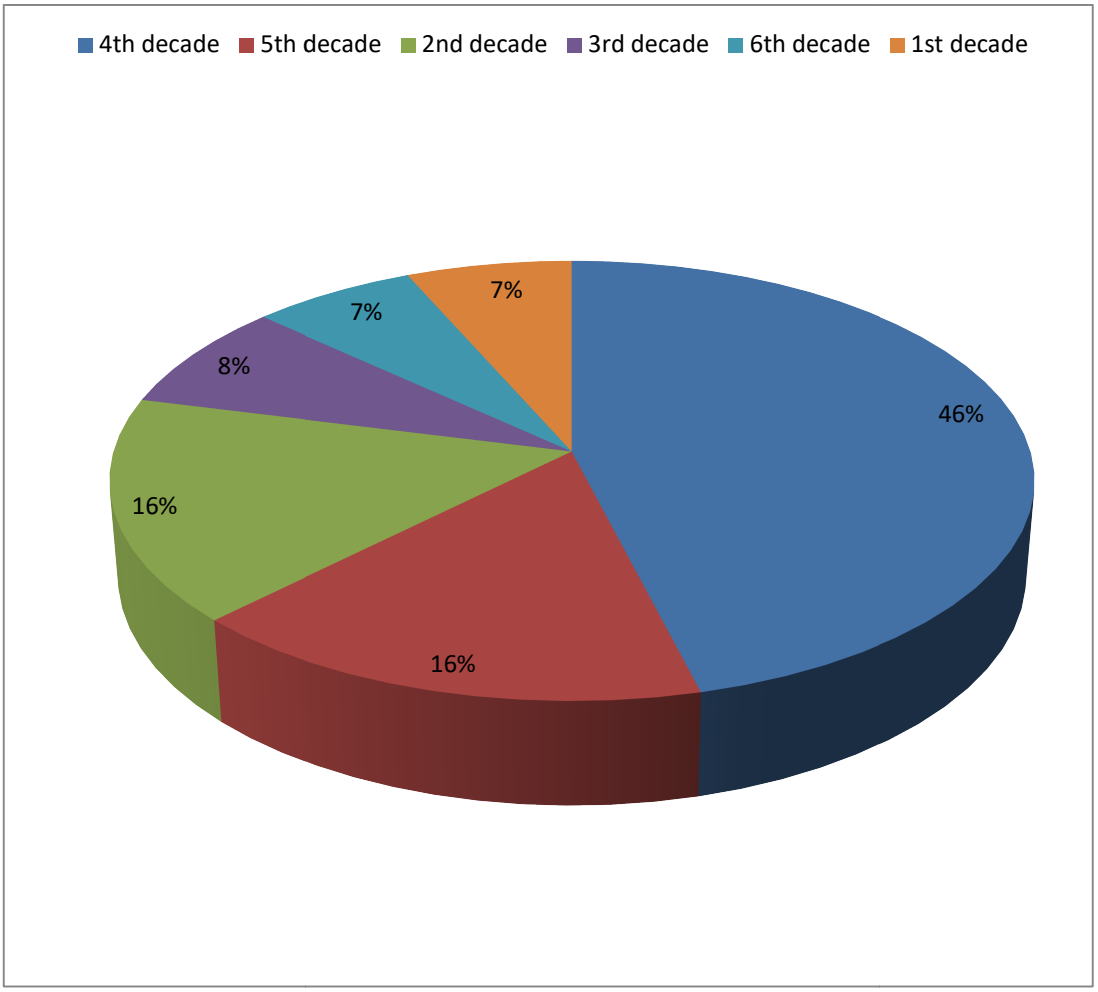


CHART 2: AGE DISTRIBUTION OF CASES

It was noted that there was high prevalence of lichen planus between 20 and 50 years of age with a peak incidence at 40 years. In children the most common age of presentation is between 2- 10 years of age.

SEX DISTRIBUTION:

On analyzing the sex distribution there was a slight female preponderance with a male to female ratio of 1:1.1. The following chart shows the sex distribution of the cases in the proposed study.

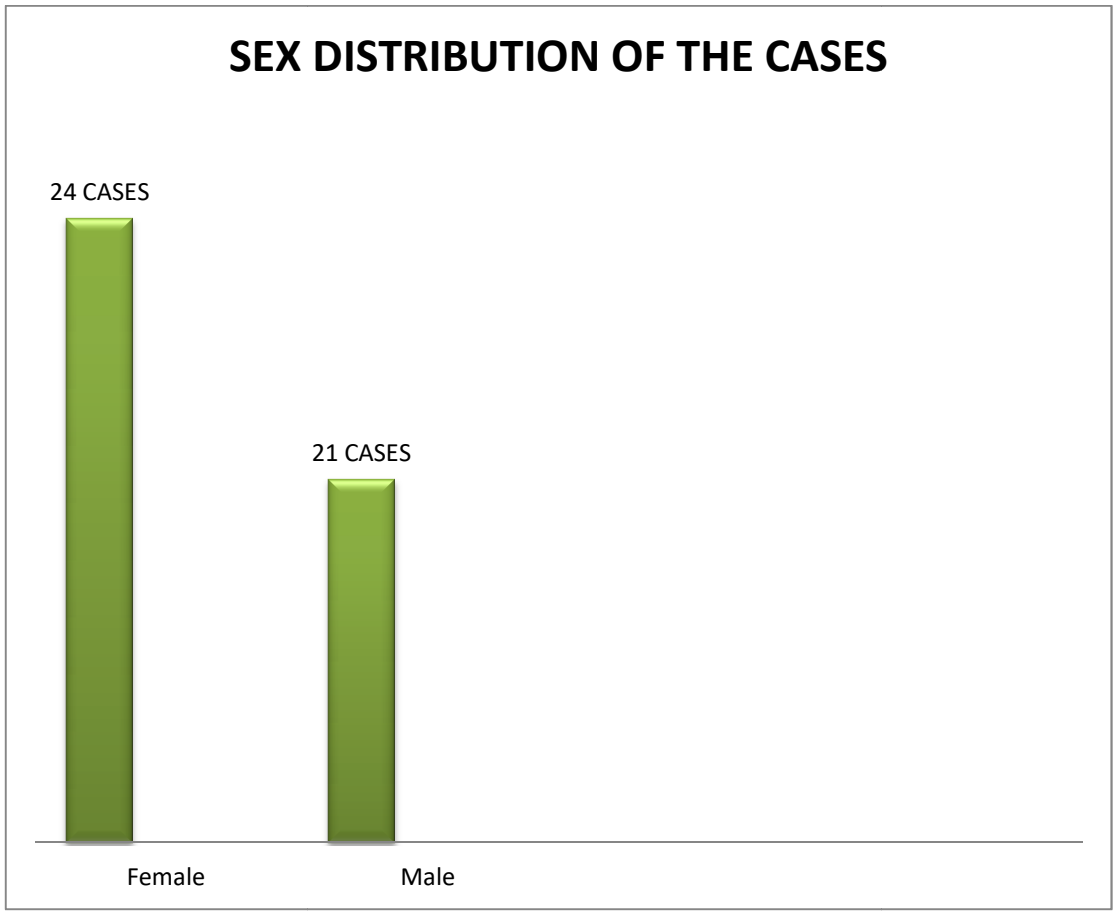


CHART 3: SEX DISTRIBUTION OF THE CASES

COMMON SITE OF THE LESION:

The various sites involved in our study is as follows in a descending order of presentation.

- Lower limbs
- Upper limbs
- Abdomen
- Chest
- Genitalia
- Oral

The following chart shows the common site of the lesion presented in our study.

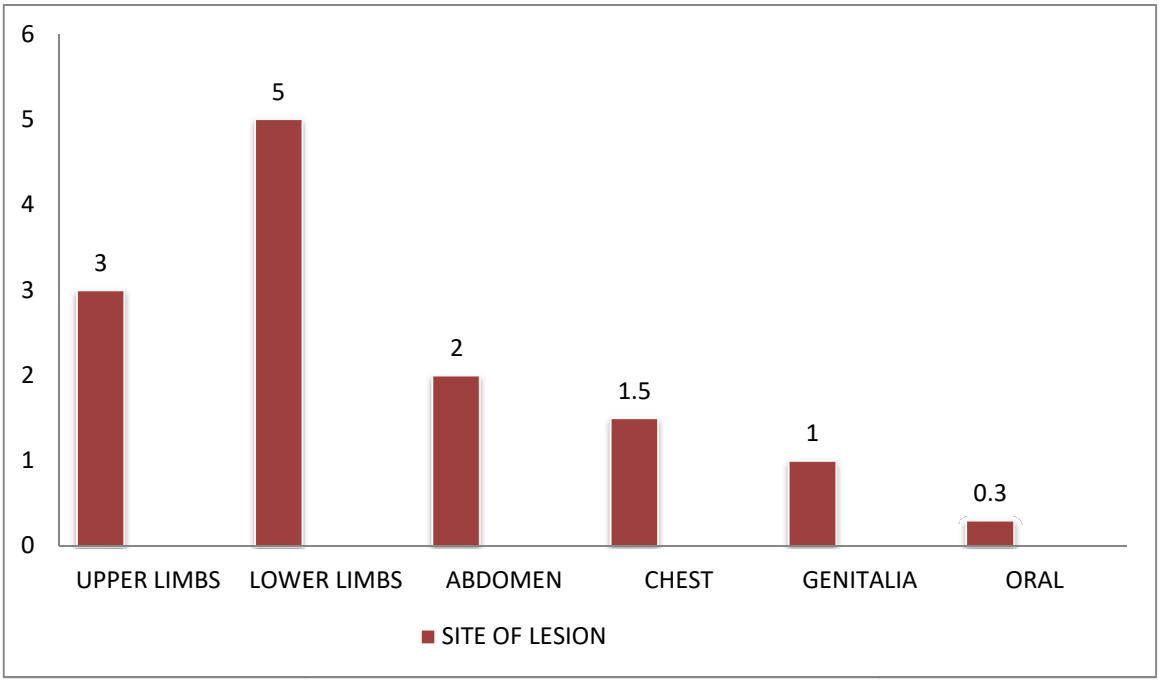


CHART 4: DISTRIBUTION OF SITES OF LESION

TABLE 2: STATISTICAL ANALYSIS- INDEPENDENT T TEST

SITES	FREQUENCY	PERCENT
ABDOMEN	2	2.2
B/L UPPER LIMBS	2	2.2
BOTH UPPER AND LOWER LIMBS	2	2.2
CHEST	2	2.2
DORSUM OF FOOT®	2	2.2
ELBOW (L)	2	2.2
GENITALIA	2	2.2
GLUTEAL REGION	2	2.2
HIPS AND THIGH	2	2.2
LOWER LIMB, TRUNK	2	2.2
LOWER LIMB(L)	4	4.4
LOWER LIMBS	26	28.9
ORAL	2	2.2
TRUNK AND UPPER LIMBS	2	2.2
UPPER AND LOWER LIMB	6	6.7
UPPER LIMB	22	24.7
VAGINA	2	2.2
TOTAL	90	100.0

Data analysis was done using independent T test and the frequency and percentage of the common site of lesions were assessed. The frequency and percentage was found to be higher with the cases presented with lesions over lower limbs which are 26 and 28.9% respectively.

EXPRESSION OF CD4 CD8 AND BCL2:

The slides were analyzed for CD4, CD8 and BCL2 IHC expression. We counted No. of lymphocytes/ 100 keratinocytes and a ratio was obtained. More than 80% of the cases had CD8 cytotoxic T lymphocytes infiltrating the Dermis. Few anti-apoptotic cells were stained by BCL2.

The following two charts (5a and 5b) highlight the CD4,CD8 and BCL2 presentation.

CHART 5A: COMPARISON OF CD4, CD8 AND BCL2 IN DERMO-EPIDERMAL JUNCTION

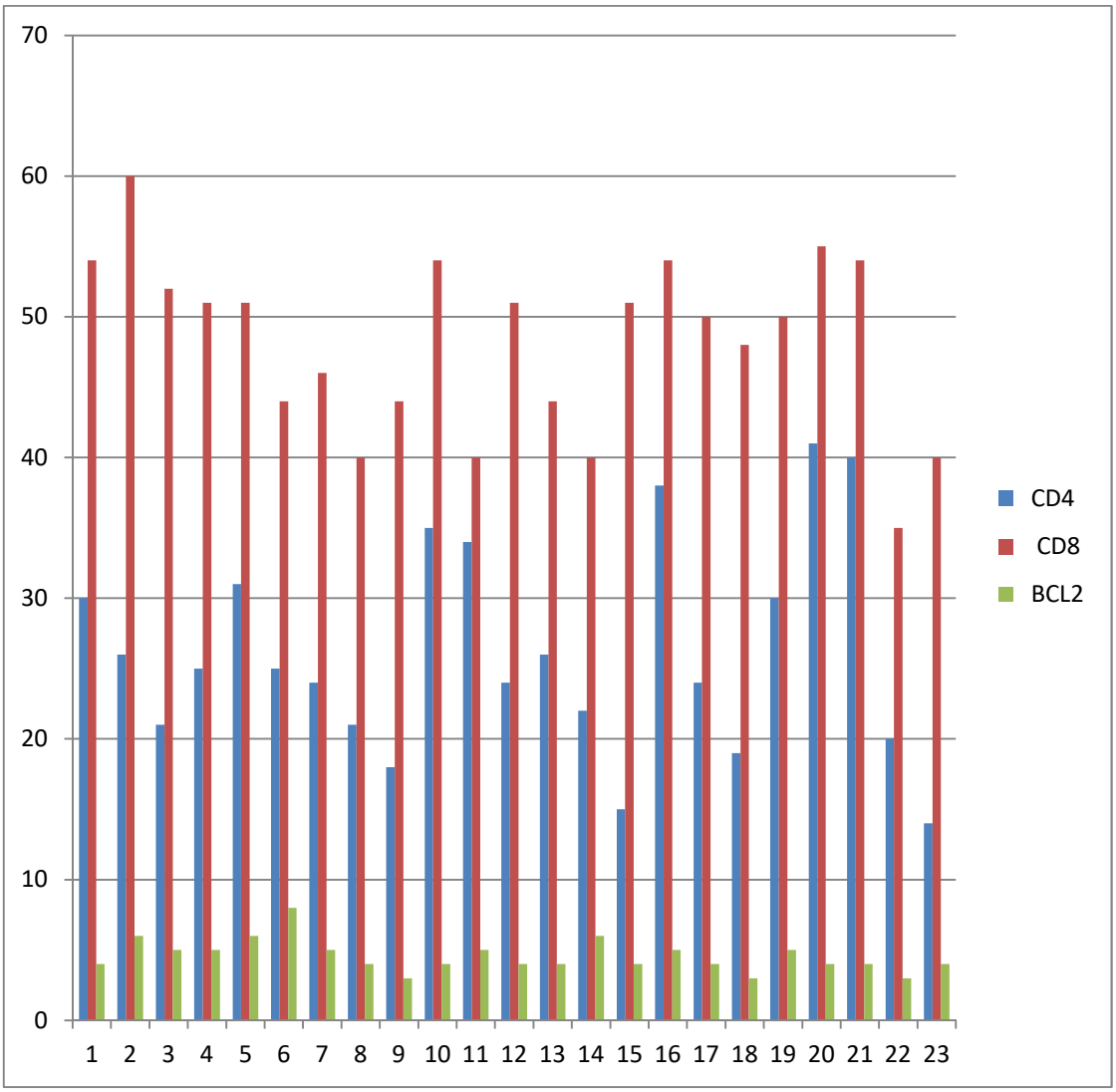
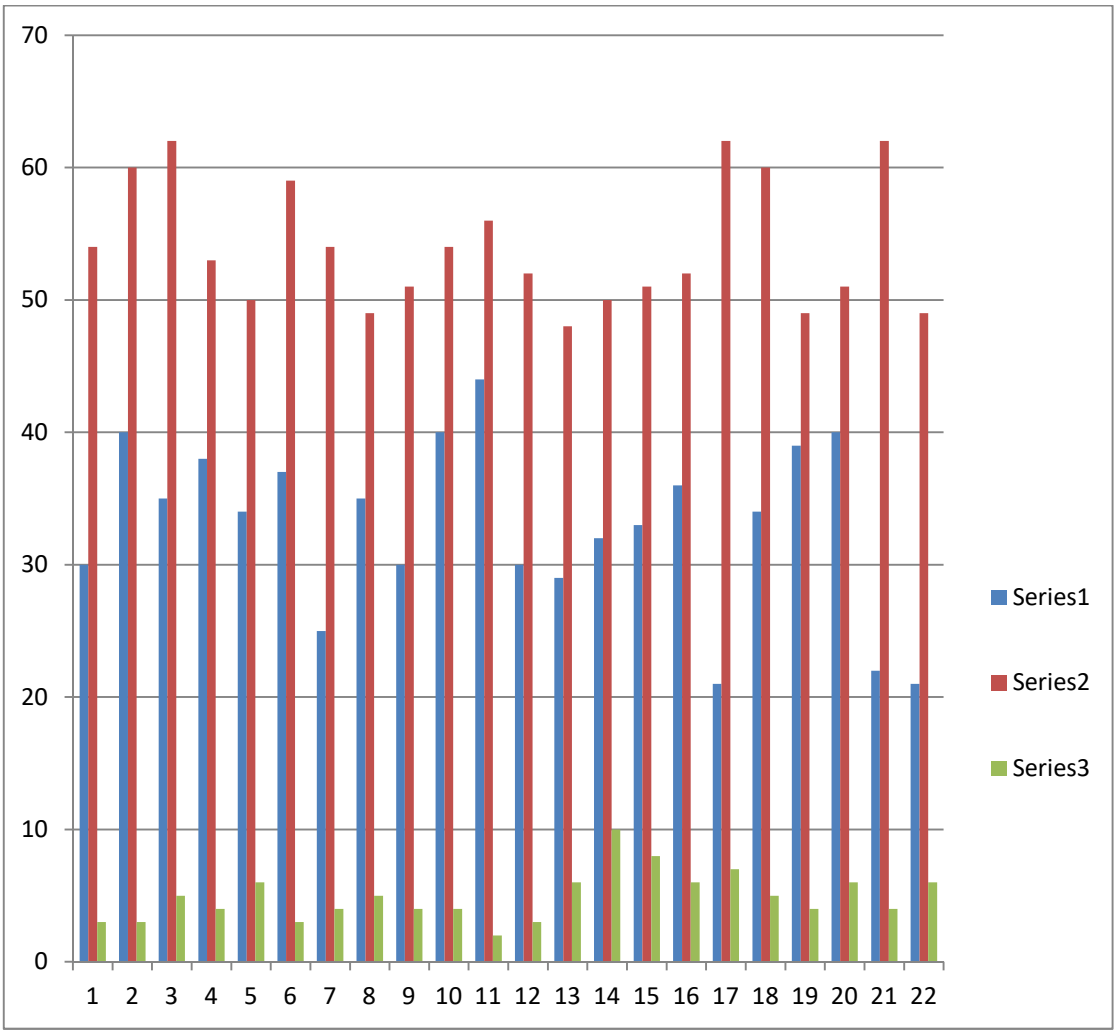


CHART 5B: COMPARISON OF CD4, CD8 AND BCL2 IN DERMO-EPIDERMAL JUNCTION



CD8 T cells were the predominant population of lymphocytes found in the lymphocytic exocytosis.CD4 T lymphocytes were comparatively less than the CD8 T lymphocytes. Anti – apoptotic cells are stained by BCL2. Chart 6 highlights the distribution of CD4 CD8 and BCL2 in the epidermis is presented in Chart 6.

CHART 6A: COMPARISON OF CD4, CD8 AND BCL2 IN THE EPIDERMIS

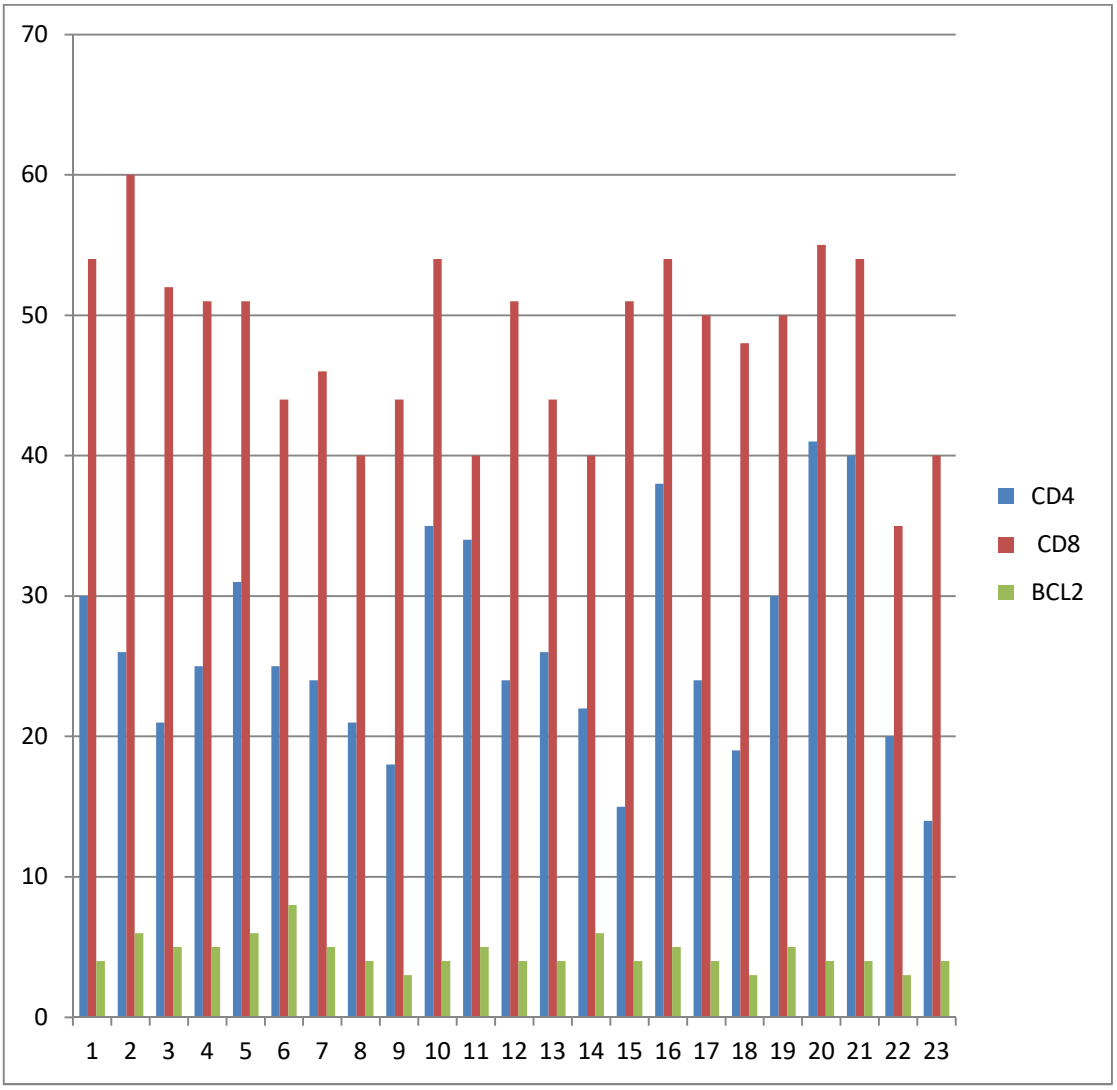


CHART 6B: COMPARISON OF CD4, CD8 AND BCL2 IN THE EPIDERMIS

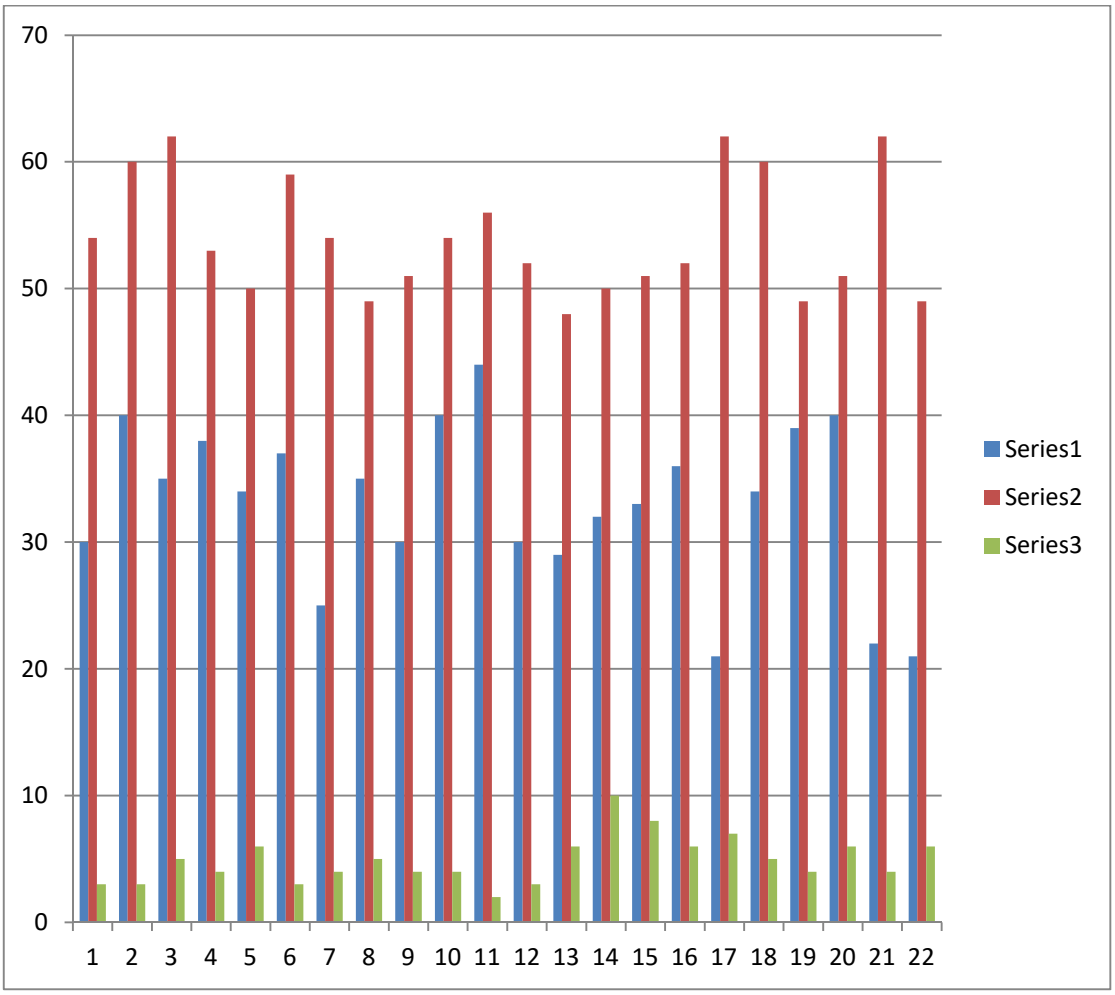


TABLE 3 : STATISTICAL ANALYSIS DATA- INDEPENDENT T TEST

LYMPHOCYTES	Groups	N	Mean	Std. Deviation	P value
CD4	1	45	29.51	7.762	.000*
	2	45	2.76	1.048	
CD8	1	45	51.02	6.265	.000*
	2	45	4.80	1.140	
BCL2	1	45	4.73	1.543	.001*
	2	45	5.89	1.511	

*-statistically significant ($p<0.05$)

Statistical analysis of the data was done using group statistics for CD4,CD8 and BCL2 positive cells. The groups were subdivided as group1 and group 2. Group 1contains the lymphocytes that were present in the epidermo-dermal junction and group 2 contains the lymphocytes that were present in the epidermis.

The lymphocytes were typed with the use of CD4, CD8 and BCL2 IHC markers. The lymphocytes stained for CD4, CD8 and BCL2 were compared between the two groups. Mean, standard deviation and p value were assessed and it was found that the values were statistically significant.

PICTURES

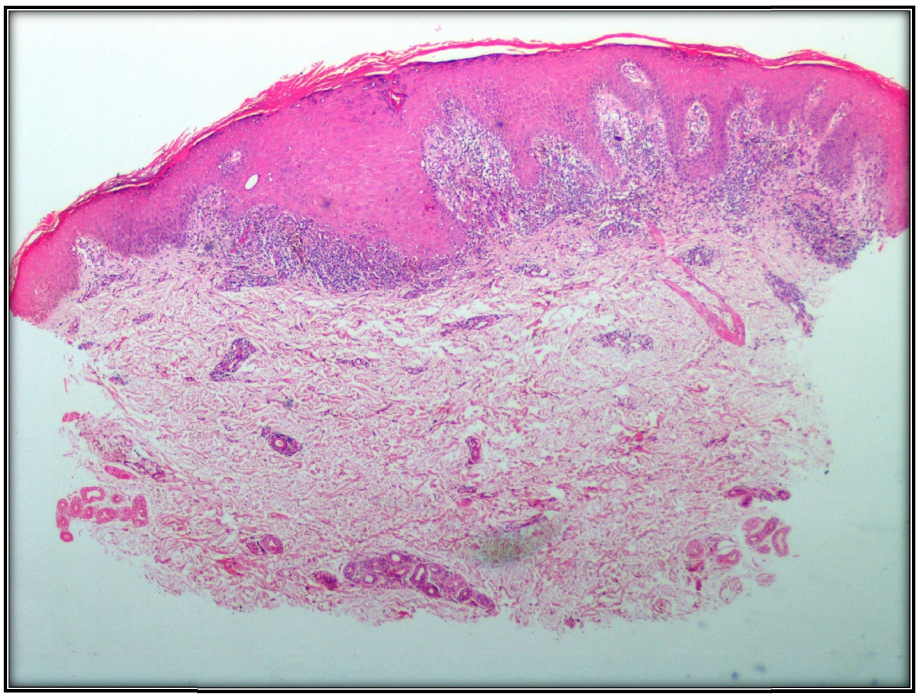


FIG 2: H&E Section :(4 X): Biopsy Of Lichen Planus Exhibiting Hyperkeratosis, Wedge Shaped Hypergranulosis, Irregular Acanthosis, Basal Cell Vacoular Damage, Dense Band Like Inflammatory Cell Infiltrate In Dermo-Epidermal Junction

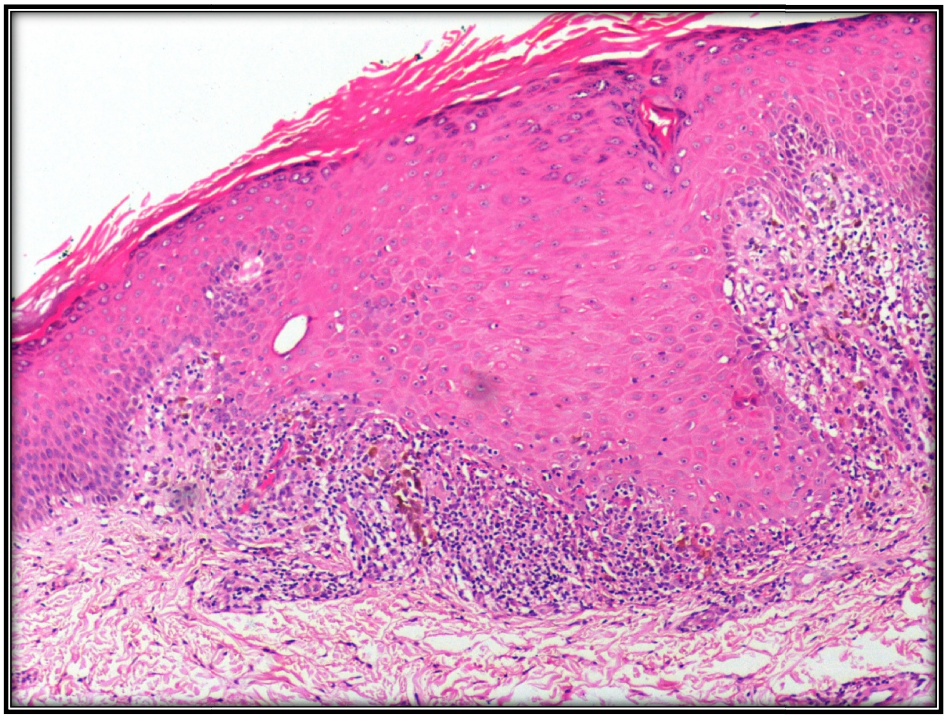
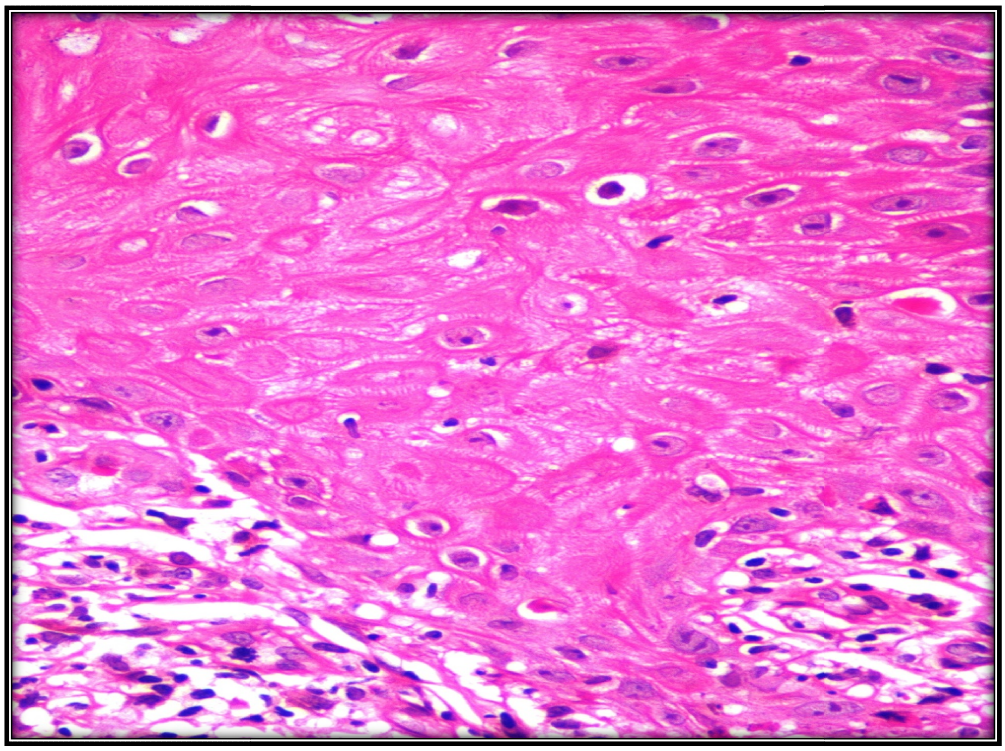


FIG 3: H&E Section :(40X): High Power Of The Section – Basal Cell Vacoular Damage And Dense Inflammatory Infiltrate In Dermo-Epidermal Junction



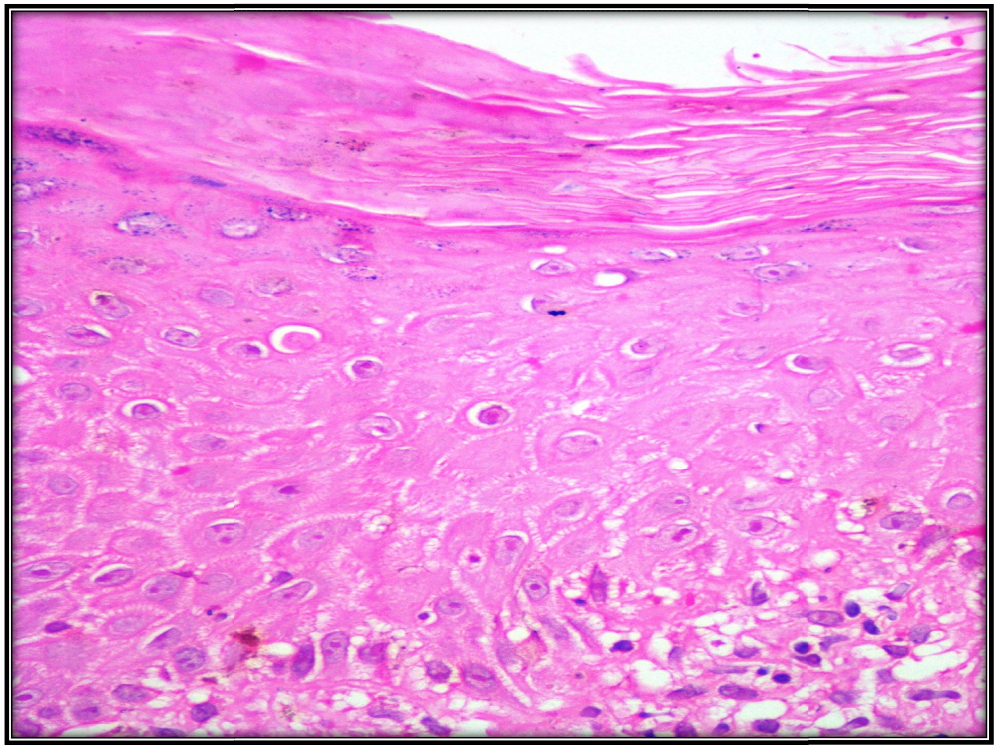


FIG 4&5: H&E Section :(40X): Shows Presence Of Civatte Bodies (Apoptotic Cells)

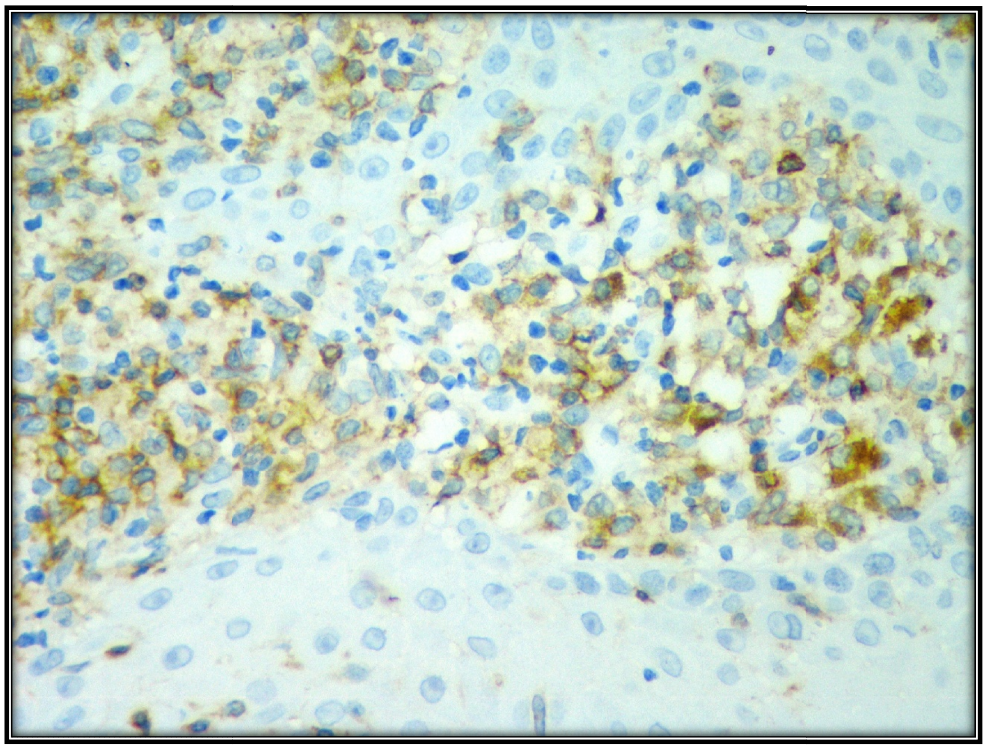


FIG 6 : IHC Marker CD4: (40x) :Positivity Of CD4 T Lymphocytes

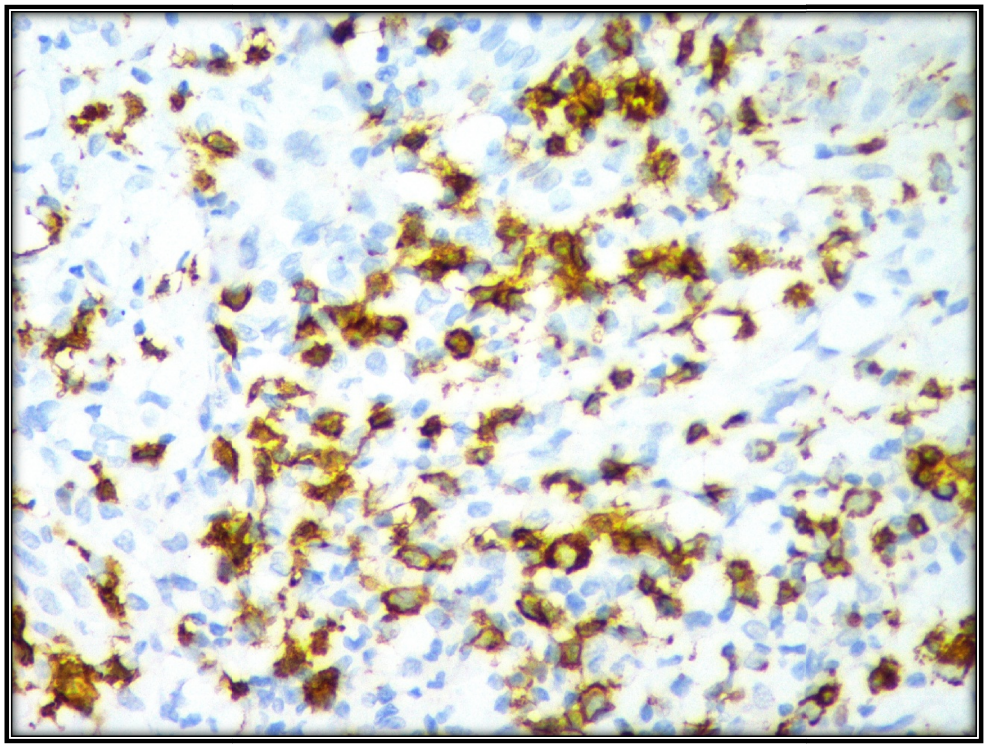


FIG 7: : IHC Marker CD8: (40x) :Positivity Of CD8 Cytotoxic T Lymphocytes

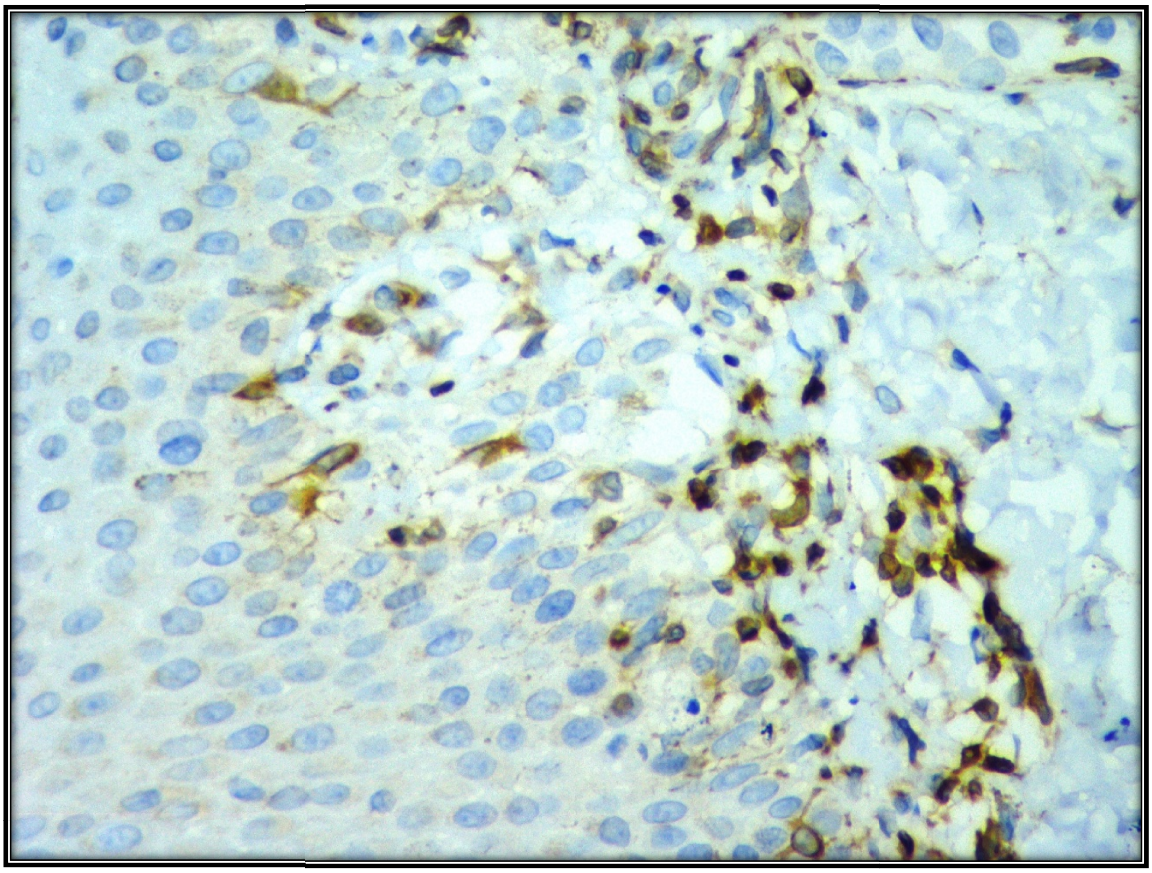


FIG 8: IHC Marker BCL2: (40x) :Positivity Of Anti-Apoptotic Cells

DISCUSSION

DISCUSSION

Lichen planus constituted 4.1 %(51 cases/ 1100 skin biopsies) among all the skin biopsies reported in our institute during the study period (Chart 1), similar to the incidence reported from the study done by Farzham Gorouhi et al.[22]

Many studies revealed that oral lichen planus has an incidence of 1-4% of the general population [50]. Soma Susan Varghese et al had done a cohort study in the Indian population. His cohort study states that the prevalence of oral lichen planus is 0.5-2.6% in the general population [51]. In our study the incidence of Oral lichen planus is 0.02% which is less when compared to other studies

From our analysis the common age group of patients was in the 4th decade (46%) followed by the 5th (16%) decade with a slight female preponderance (Chart 2 and 3). This is similar to the earlier studies done by M.Ammar et al and P.B Sugerman et al [52-53].

Lichen planus can affect any part of the body surface. Literature states that lichen planus is most often seen on the volar aspect of the wrist and around the ankles. Analyses done by Richard.P.Usatine et al found that the common presentations of the lesions in the cases were noted in the wrist, forearms and the lower extremities [54]. From our study we infer that the lower limb lesions were more common (Chart 4),which is similar the above quoted literatures.

The cell mediated immunity plays an important role in the pathogenesis of lichen planus. The sub epithelial infiltrate consists mainly of the T cells

including the T helper cells (CD4 cells) and suppressor cytotoxic T cells (CD8 T cells). The ratio of CD4:CD8 in the sub epithelial infiltrate is 2:1, whereas after the inflammatory process the CD8 T cells gets activated and the ratio gets altered[7].

Identification of the type of lymphocytic infiltration and its relation with lymphocytic exocytosis and apoptosis has changed the algorithm of treatment approach to lichen planus. Some of the prevailing local factors indicated by our biopsies including the increasing incidence, female preponderance, age at presentation, site of the lesions and chronicity of the disease prompted us to do this study. This study helps us to understand the pathogenesis of lichen planus in detail and to analyze the findings given in the literatures.

An extensive search in the literature on Indian studies for CD4 CD8 AND BCL2 expression in lichen planus indicated a paucity of literature. Therefore, we considered it to be important for us to do this study.

We identified and retrieved paraffin blocks of 45 cases of lichen planus from archives of Pathology using exclusion and inclusion criteria as discussed earlier. In our study group of 45 cases, the predominant type of lichen planus was cutaneous type. And we found a significant case presented with oral lichen planus.

Initially the main attention was on the CD4 helper T lymphocytes due to their increased predominance. But later the concepts of approach towards the pathogenesis of lichen planus have been changed and the recent literatures focus mainly on the CD8 T lymphocytes and its role.

CD8 T cells acts by recognizing an antigen associated with MHC class I molecule that is present on the lesional keratinocytes. This results in death of the keratinocytes by apoptosis. In both oral and cutaneous lichen planus CD8 cells predominate in the epithelial and sub epithelial compartments.

Study done by Eman Nofal et al stated that lichen planus is a chronic inflammatory disease that is related to cell-mediated immunity [55]. Histologically, LP is characterized by hyperkeratosis, liquefaction degeneration of the basal cell layer, presence of Civatte bodies and apoptosis has been reported and it was suggested that the civatte bodies represent non-phagocytosed apoptotic cell fragments.

In lichen planus the apoptotic mechanisms are blocked by the proto-oncogene BCL2. BCL2 is an anti apoptotic membrane associated molecule that resides in the nuclear envelope and mitochondria. The strong expression of BCL2 in dermal lymphocytes before treatment inhibits the apoptosis in lymphocytes that strengthens cell-mediated immune process causing chronicity of the lesion.

The low expression of BCL2 in lymphocytes after treatment may provide evidence that apoptosis of lymphocytes is an important mechanism of the therapeutic action of NB-UVB in LP [55].

After gaining knowledge from various literatures we went ahead with our study. We typed the lymphocytes using CD4, CD8 and anti apoptotic cells using BCL2 IHC markers.

From our study, we infer that CD8 cytotoxic T lymphocytes (Fig 7) were increased in the epithelial and sub epithelial compartments. We also observed that in the cases were CD8 cells were increased there was a concordant increase in the apoptotic cells also. Hence this proves the cytotoxic nature of the CD8 cells and their relation with apoptosis.

Sections were BCL2 marker (Fig 8) was significantly present showed increase in lymphocytic infiltrate in the Dermo-epidermal junction. Therefore the presence of anti-apoptotic cells were directly proportional to the inflammatory infiltrates.

We analyzed our results statistically by using Independent T test with the help of SPSS 23.0 software (Table 3). The standard deviation of CD4 is 7.762 and 1.048, CD8 is 6.265 and 1.140 and BCL2 is 1.543 and 1.511. The p values obtained were 0.00, 0.00 and 0.001. The SD and p value obtained were statistically significant.

From the above findings we infer that CD8 cells are the predominant population of lymphocytes in LP (as seen in chart 5 and 6) and are directly proportional to the increase in apoptotic cells. BCL2 expression shows the presence of anti-apoptotic cells and it is directly proportional to increase in inflammatory infiltrate in LP. The above described mechanisms are important in the pathogenesis of LP.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In conclusion, our study on Lichen planus reported in our Institute from June 2014- December 2016 revealed that,

- The incidence of Lichen Planus in our study was 4.1%.
- The mean age group of presentation was 4^{th} decade.
- There was a slight female preponderance.
- The common site of the lesion presented was the lower extremities.
- CD8 cytotoxic T lymphocytes were prevalent with a concordant increase in the apoptotic cells.
- Anti –apoptotic cells highlighted by BCL2 were directly proportional to the increase in chronic inflammatory response.

BIBLIOGRAPHY

REFERENCES

1. De Panfilis G, Manara G , Ferrari C, Manfredi G, Allegra F. Imbalance in phenotypic expression of T cell subpopulations during different evolutional stages of lichen planus lesions. Acta Derm Venereol 1983; 63:369.

2. Neppelberg E, Johannessen AC, Jonsson R. Apoptosis in oral lichen planus. Eur J Oral Sci 2001; 109:361.

3. Weedon D. Apoptosis in lichen planus. Clin Exp Dermatol 1976; 5: 425

4. Miller CS, Epstein JB, Hall EH, Sirois D. Changing oral care needs in the United States: the continuing need for oral medicine. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001; 91:34-44

5. James W Patterson. The lichenoid reaction pattern (‘interface dermatitis’). In, Churchill Livingstone (ed). Weedons Skin Pathology, 4th edition.USA, 2016; 37-80

6. Barbara Young, Geraldine O’Dowd, Phillip Woodford.Skin. In, Churchill Livingstone(ed). Wheaters Functional Histology. A Text and Colour Atlas, 6th edition. Australia, 2016; 159-179.

7. David E. Elder.Histology of the skin.In, Wolters Kluwer(ed). Lever’s Histopathology of the skin, 11th edition. Philadelphia, 2015; 8-75

8. Holbrook KA, Odland GF. The fine structure of developing human epidermis: light, scanning, and transmission electron microscopy.

9. Penneys NS, Fulton JE Jr, Weinstein GD, et al. Location of proliferating cells in human epidermis. Arch Dermatol 1970; 101(3):323-327

10. Amagai M, Klaus-Kovtun V, Stanley JR.Autoantibodies against a novel epithelial cadherin in pemphigous vulgaris, a disease of cell adhesion. Cell 1991; 67(5):869-877.

11. Shimizu H, Masunaga T, Ishiko A, et al. Demonstration of desmosomal antigens by electron microscopy using cryofixed and cryosubmitted skin with silver- enhanced gold probe. J Histochem Cytochem 1994; 42(5): 687-692.

12. Lazarus GS, Hatcher VB, Levine N.Lysosomes and the skin. J Invest Dermatol 1975; 65(3):259-271.

13. Christophers E. Cellular architecture of the stratum corneum. J Invest Dermatol 1971; 56(3):165-169.

14. Pearse AGE. Histochemistry: theoretical and applied, 3rd ed. Edinburgh, Scotland: Churchill Livingstone, 1972.

15. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965; 19(6):739-744.

16. Moll I. Merkel cell distribution in human hair follicles of the fetal and adult scalp. Cell Tissue Res 1994; 277(1):131-138

17. Di Como CJ, Urist MJ, Babayan I et al. p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002; 8(2):494-501.

18. Fitzpatrick TB. Human melanogensis. Arch Dermatol Syph 1952; 65:379-391

19. T. Shiohara and Y. Kano, “Lichen Planus and lichenoid dermatoses,”in *Dermatology*, J. L. Bolognia, J. Jorizzo, and R. P.Rapini, Eds., pp. 159–180, Mosby Elsevier, New York, NY,USA,2008.

20. C. S.Miller, J. B. Epstein, E. H. Hall, and D. Sirois, “Changing oral care needs in the United States: the continuing need for oral medicine,” *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, vol. 91, no. 1, pp. 34–44, 2001

21. Luis-Montoya P, Dominguez-Soto L, Vega-Memije E. Lichen planus in 24 children with review of the literature. Pediatr Dermatol 2005; 22(4):295–8.

22. Farzam Gorouhi, Parastoo Davari, and Nasim Fazel. “Cutaneous and Mucosal Lichen Planus: A Comprehensive Review of Clinical Subtypes, Risk Factors, Diagnosis, and Prognosis,” The Scientific World Journal Volume 2014. 22pg

23. F. Gorouhi, A. Firooz, A. Khatami et al., “Interventions forcutaneous Lichen Planus,” Cochrane Database of Systematic Reviews, no. 4, Article ID CD008038, 2009.

24. J.P. Welsh, C. B. Skvarka, and H. B. Allen, “A novel visual clue for the diagnosis of hypertrophic Lichen Planus,” *Archives of Dermatology*, vol. 142, no. 7, p. 954, and 2006

25. Gawkrodger DJ, Stavropoulos PG, McLaren KM, Buxton PK. Bullous lichen planus and lichenplanus pemphigoides: clinico-pathological comparisons. Clin Exp Dermatol 1989; 14(2):150–3.

26. E. Tan, M.Martinka,N. Ball, and J. Shapiro, "Primary cicatricial alopecias: clinic pathology of 112 cases," *Journal of the American Academy of Dermatology*, vol. 50, no. 1, pp. 25–32, 2004.

27. P. Assouly and P. Reygagne, "Lichen planopilaris: update on diagnosis and treatment," *Seminars in Cutaneous Medicine and Surgery*, vol. 28, no. 1, pp. 3–10, 2009.

28. Nakamura R, Broce AA, Palencia DP, Ortiz NI, Leverone A. Dermatoscopy of nail lichenplanus. Int J Dermatol 2013; 52(6):684–7.

29. Tosti A, Peluso AM, Fanti PA, Piraccini BM. Nail lichen planus: clinical and pathologic study of twenty-four patients. J Am Acad Dermatol 1993; 28(5 Pt 1):724–30.

30. Brauns B, Stahl M, Schon MP, Zutt M. Intralesional steroid injection alleviates nail lichen planus. Int J Dermatol 2011; 50(5):626–7

31. F. A. de Sousa and L. E. B. Rosa, "Oral Lichen Planus: clinical and histopathological considerations," *Brazilian Journal of Otorhinolaryngology*, vol. 74, no. 2, pp. 284–292, 2008.

32. J. J.Thorn, P. Holmstrup, J. Rindum, and J. J. Pindborg, “Course of various clinical forms of oral Lichen Planus. A prospective follow-up study of 611 patients,” *Journal of Oral Pathology*, vol.17, no. 5, pp. 213–218, 1988.

33. N. Mollaoglu, “Oral Lichen Planus: a review,” *British Journal of Oral andMaxillofacial Surgery*, vol. 38, no. 4, pp. 370–377, 2000.

34. T. McPherson and S. Cooper, “Vulval lichen sclerosus and Lichen Planus,” *Dermatologic Therapy*, vol. 23, no. 5, pp. 523–532, 2010.

35. F. M. Lewis, “Vulval Lichen Planus,” *British Journal of Dermatology*, vol.138, no. 4, pp. 569–575, 1998.

36. A. L. O. Helgesen, P. Gjersvik, P. Jebsen, R. Kirschner, and T.Tanbo, “Vaginal involvement in genital erosive Lichen Planus,”*Acta Obstetricia et Gynecologica Scandinavica*, vol. 89, no. 7, pp.966–970, 2010.

37. L. P. Fox, C. J. Light dale, and M. E. Grossman, “Lichen Planus of the esophagus: what dermatologists need to know,” *Journal of the American Academy of Dermatology*, vol. 65, no. 1, pp. 175–183, 2011.

38. V. Kumar, A. Abbas, and J. Aster, *Robbins & Cotran Pathologic Basis of Disease*, Saunders, Philadelphia, Pa, USA, 8th edition, 2009

39. Bramanti TE, Dekker NP, Lozada-Nur F, Sauk JJ, Regezi JA. Heat shock (stress) proteins and gamma delta T lymphocytes in oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 80(6):698–704

40. Sugerman PB, Savage NW, Walsh LJ, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med 2002; 13(4):350–65.

41. Zhao ZZ, Sugerman PB, Zhou XJ, Walsh LJ, Savage NW. Mast cell degranulation and the role of T cell RANTES in oral lichen planus. Oral Dis 2001; 7(4):246–51.

42. Hasseus B, Jontell M, Brune M, Johansson P, Dahlgren UI. Langerhans cells and T cells in oral graft versus host disease and oral lichen planus. Scan J Immunol 2001; 54(5):516–24.

43. Lu R, Zhang J, Sun W, Du G, Zhou G. Inflammation-related cytokines in oral lichen planus: an overview. J Oral Pathol Med 2013.

44. Kulthanan K, Jiamton S, Varothai S, Pinkaew S, Sutthipinittharm P. Direct immunofluorescence study in patients with lichen planus. Int J Dermatol 2007;46(12):1237–41

45. Ragaz A, Ackerman AB. Evolution, maturation, and regression of lesions of lichen planus. New observations and correlations of clinical and histologic findings. Am J Dermatopathol1981; 3(1):5–25.

46. Martin MD, Broughton S, Drangsholt M. Oral lichen planus and dental materials: a case–control study. Contact Dermatitis 2003;48(6):331–6

47. Stoopler ET, Parisi E, Sollecito TP. Betel quid-induced oral lichen planus: a case report. Cutis 2003; 71(4):307–11

48. Khan A, Farah CS, Savage NW, Walsh LJ, Harbrow DJ, Sugerman PB (2001). Cytokines in oral lichen planus. *J Oral Pathol Med* (submitted).

49. Steinman RM, Turley S, Mellman I, Inaba K (2000). The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med* 191:411-416.

50. Christopher Griffiths. Rook's Textbook of Dermatology. In, Wiley Blackwell(ed) 9th edition, volume 2 UK 2010; 37.1-37.20

51. Soma Susan Varghese, Giju Baby George et al. Epidemiology of Oral lichen planus in a cohort of south Indian population: A Reterospective study. Journal of Cancer prevention 2016: 21:55

52. M.Ammar, M.Mokni, Boubaker S, EI Gaied A, Ben Osman A, Louzir H. Involvement of granzyme B and granulysin in the cytotoxic response in lichen planus. J Cutan Pathol 2008; 35: 630-634

53. P.B Sugarman et al. The pathogenesis of Oral lichen planus. Crit Rev Oral Biol Med 2002; 13(4): 350-365

54. Richard P Usatine MD et al. Diagnosis and treatment of Lichen Planus. Am Fam Physician 2011: 84(1):53-60

55. Eman Nofal, Masda Assaf and Kamal A.El Kashishy et al. Egyptian Dermatology Online Journal 2008: 4(2):2

MASTER CHART

SL.NO	SEX	BIOPSY SITE	DERMO-EPIDERMAL JUNCTION (No. of lymphocytes/100 keratinocytes)		
			CD4	CD8	BCL2
1	F	UPPER LIMB	30	54	4
2	M	UPPER LIMB	26	60	6
3	M	GLUTEAL REGION	21	52	5
4	M	UPPER AND LOWER LIMB	25	51	5
5	M	ABDOMEN	31	51	6
6	F	ELBOW (L)	25	44	8
7	M	UPPER LIMB	24	46	5
8	M	DORSUM OF FOOT®	21	40	4
9	F	LOWER LIMB	18	44	3
10	M	B/L LOWER LIMBS	35	54	4
11	F	UPPER LIMB	34	40	5
12	F	UPPER LIMB	24	51	4
13	F	ABDOMEN	26	44	4
14	M	ABDOMEN	22	40	6
15	F	LOWER LIMB	15	51	4
16	F	UPPER LIMB	38	54	5
17	M	LOWER LIMB, TRUNK	24	50	4
18	F	LOWER LIMB	19	48	3
19	F	GENITALIA	30	50	5
20	M	UPPER AND LOWER LIMB	41	55	4
21	M	LOWER LIMB(L)	40	54	4
22	F	LOWER LIMB, TRUNK	20	35	3
23	F	UPPER LIMB	14	40	4
24	F	UPPER LIMB	30	54	3
25	M	LOWER LIMB	40	60	3
26	F	UPPER AND LOWER LIMB	35	62	5

SL.NO	SEX	BIOPSY SITE	DERMO-EPIDERMAL JUNCTION (No. of lymphocytes/100 keratinocytes)		
			CD4	CD8	BCL2
27	F	HIPS AND THIGH	38	53	4
28	F	LOWER LIMBS	34	50	6
29	F	VAGINA	37	59	3
30	F	UPPER LIMBS	25	54	4
31	F	UPPER LIMBS	35	49	5
32	F	CHEST	30	51	4
33	M	LOWER LIMB	40	54	4
34	M	LOWER LIMB	44	56	2
35	F	LOWER LIMB	30	52	3
36	M	LOWER LIMBS	29	48	6
37	F	LOWER LIMBS	32	50	10
38	M	BOTH UPPER AND LOWER LIMBS	33	51	8
39	M	TRUNK AND UPPER LIMBS	36	52	6
40	M	ORAL	21	62	7
41	F	B/L UPPER LIMBS	34	60	5
42	F	LOWER LIMBS	39	49	4
43	M	UPPER LIMBS	40	51	6
44	M	LOWER LIMBS	22	62	4
45	M	LOWER LIMBS	21	49	6

SI.NO	AGE	SEX	BIOPSY SITE	INTRA-EPITHELIAL LYMPHOCYTES(No of lymphocytes/ 100 keratinocytes)			Apoptotic cells
				CD4	CD8	BCL2	
1	26	F	UPPER LIMB	4	7	10	6
2	62	M	UPPER LIMB	5	8	9	9
3	51	M	GLUTEAL REGION	3	5	5	6
4	2	M	UPPER AND LOWER LIMB	2	4	6	5
5	10	M	ABDOMEN	4	5	7	7
6	42	F	ELBOW (L)	3	4	4	4
7	50	M	UPPER LIMB	3	5	6	5
8	34	M	DORSUM OF FOOT®	4	7	8	4
9	14	F	LOWER LIMB	3	6	8	7
10	44	M	B/L LOWER LIMBS	4	5	7	8
11	8	F	UPPER LIMB	5	6	6	5
12	19	F	UPPER LIMB	2	4	5	6
13	20	F	ABDOMEN	1	5	7	5
14	57	M	ABDOMEN	3	5	6	5
15	12	F	LOWER LIMB	2	4	5	3
16	50	F	UPPER LIMB	4	6	9	
17	46	M	LOWER LIMB, TRUNK	3	5	6	3
18	43	F	LOWER LIMB	1	4	5	6
19	67	F	GENITALIA	2	5	7	
20	42	M	UPPER AND LOWER LIMB	4	6	6	2
21	9	M	LOWER LIMB(L)	3	7	7	4
22	23	F	LOWER LIMB, TRUNK	2	3	5	5
23	58	F	UPPER LIMB	3	4	4	6
24	8	F	UPPER LIMB	2	3	4	6
25	66	M	LOWER LIMB	4	5	5	10
26	65	F	UPPER AND LOWER LIMB	3	3	4	5

SI.NO	AGE	SEX	BIOPSY SITE	INTRA-EPITHELIAL LYMPHOCYTES(No of lymphocytes/ 100 keratinocytes)			Apoptotic cells
				CD4	CD8	BCL2	
27	50	F	HIPS AND THIGH	2	4	5	4
28	64	F	LOWER LIMBS	2	5	6	6
29	21	F	VAGINA	3	5	5	5
30	45	F	UPPER LIMBS	4	5	7	5
31	65	F	UPPER LIMBS	3	4	5	4
32	37	F	CHEST	2	5	5	6
33	52	M	LOWER LIMB	1	4	5	5
34	9	M	LOWER LIMB	2	4	6	6
35	36	F	LOWER LIMB	3	4	5	7
36	21	M	LOWER LIMBS	2	5	5	5
37	40	F	LOWER LIMBS	3	4	4	5
38	36	M	BOTH UPPER AND LOWER LIMBS	2	3	3	5
39	36	M	TRUNK AND UPPER LIMBS	1	4	5	6
40	34	M	ORAL	1	5	6	8
41	53	F	B/L UPPER LIMBS	2	5	7	9
42	45	F	LOWER LIMBS	3	3	4	4
43	56	M	UPPER LIMBS	4	5	6	5
44	14	M	LOWER LIMBS	3	6	8	9
45	16	M	LOWER LIMBS	2	5	7	5

ABBREVIATIONS

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- LP – Lichen Planus
- OLP- Oral Lichen Planus
- CD- Cluster Differentiation
- UV light – Ultra Violet radiation
- HIV – Human Immunodeficiency Virus
- HCV – Hepatitis C Virus
- NOD- like receptors- Nucleotide-binding oligomerization Domain- like receptors
- NK-cells – Natural Killer Cells
- MHC – Major Histocompatibility Complex
- IFN-gamma – Interferon – gamma
- IL 17 – Interleukin 17
- BCL2 protein- B- Cell Lymphoma-2
- TNF- Tumor Necrosis Factor
- FADD-Fas- Associated Death Domain
- ICAM- Intercellular Adhesion Molecule